

Nils Dahlgren

The Control of Cerebral Blood Flow
and Oxygen Consumption During Normal
and Increased Carbon Dioxide Tensions:
Influence of Monoamines and Prostaglandins

Lund 1981



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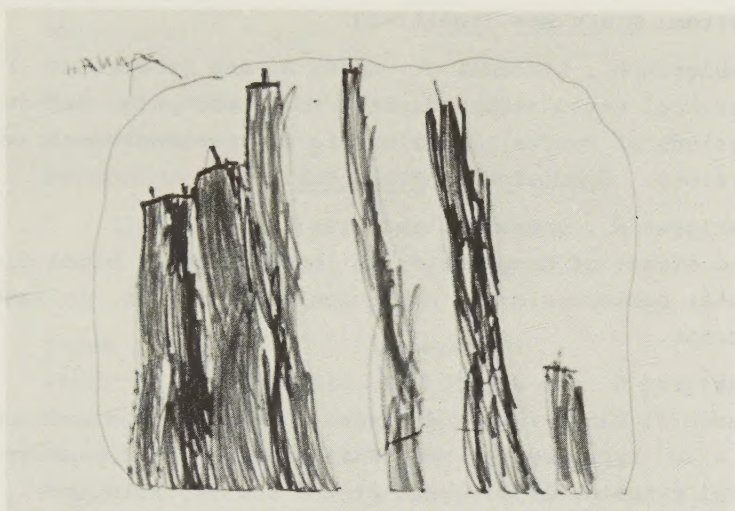
THE CONTROL OF CEREBRAL BLOOD FLOW AND OXYGEN
CONSUMPTION DURING NORMAL AND INCREASED CARBON DIOXIDE
TENSIONS: INFLUENCE OF MONOAMINES AND PROSTAGLANDINS

NILS DAHLGREN

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Local CBF for some brain structures. Values are given as means $\pm$ S.E.M. For further information please contact Hanna Dahlgren (5 years old), Lund.

To Karin
Hanna, Louise and Eric

The present thesis is based on the following papers:

- I. Dahlgren N., Lindvall O., Sakabe T. and Siesjö B.K. (1981)
Cerebral blood flow and oxygen consumption in the rat brain after lesions of the noradrenergic locus coeruleus system. Brain Res. 209:11-23.
- II. Dahlgren N., Lindvall O., Nobin A. and Stenevi U. (1981)
Cerebral circulatory response to hypercapnia: Effects of lesions of central dopaminergic and serotonergic neuron systems. Submitted to Brain Res.
- III. Dahlgren N., Ingvar M. and Siesjö B.K. (1981)
The effect of propranolol on local cerebral blood flow under normocapnic and hypercapnic conditions. In manuscript.
- IV. Dahlgren N. and Siesjö B.K. (1981)
Cerebral blood flow and oxygen consumption in normocapnia and hypercapnia: Modulating influence of paravertebral sympathetic blockade at the low thoracic level. Submitted to Acta Anesthesiol.Scand.
- V. Dahlgren N., Nilsson B., Sakabe T. and Siesjö B.K. (1981)
The effect of indomethacin on cerebral blood flow and oxygen consumption in the rat at normal and increased carbon dioxide tensions.
Acta Physiol.Scand. (In press).
- VI. Dahlgren N. and Siesjö B.K. (1981)
Effects of indomethacin on cerebral blood flow and oxygen consumption in barbiturate-anesthetized normocapnic and hypercapnic rats. J.CBF and Metabol. (Accepted)
- VII. Dahlgren N., Ingvar M., Yokoyama H. and Siesjö B.K. (1981)
The effect of indomethacin on local cerebral blood flow in awake, minimally restrained rats. J.CBF and Metabol. (Accepted)

In the text the papers are referred to by their Roman numerals.

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INTRODUCTION

The brain, an organ whose cells are continuously active, has unusual energy demands. As a result, the proper functioning of brain cells requires a constant supply of oxygen and substrate. Since the capacity of the tissue to store glucose, the main substrate utilized, is limited and, since the oxygen stores are virtually nil, normal brain function is critically dependant upon the adjustment of cerebral blood flow (CBF) to match the metabolic and functional requirements. It is not surprising, therefore, that the mechanisms coupling metabolism and blood flow have been intensely studied.

Most of our present knowledge concerning CBF regulation dates back to 1945, when Kety and Schmidt presented their method for indirect measurement of CBF, as well as of cerebral metabolic rate for oxygen (CMR_{O_2}) and glucose (CMR_{gl}). The Kety-Schmidt technique, which can be applied to man without the use of anaesthetic procedures, was subsequently adapted to anaesthetized animals. In some of these applications, cerebral venous blood is sampled from the superior sagittal sinus, allowing derivation of cerebral cortical blood flow and metabolic rate (Homburger et al. 1946, Theye and Michenfelder 1968, Norberg and Siesjö 1974). However, other techniques have allowed quantitative assessment of regional and local CBF (and metabolic rate). In 1961, Lassen and Ingvar introduced their technique for regional blood flow determination. The subsequent application of this technique in man allowed the study of perfusion rates in more circumscribed brain regions. Further methodological development has permitted measurements not only of regional CBF but of regional CMR_{O_2} as well (Terpogossian et al. 1970, Raichle et al. 1976).

In experimental animals, methods have become available for measurement of local CBF (Landau et al. 1955, Reivich et al. 1969, Sakurada et al. 1978) and CMR_{gl} (Sokoloff et al. 1977). These techniques, which utilize quantitative autoradiography for measurement of tracer uptake, allow resolution of CBF and CMR_{gl} changes at the local tissue level. Lately, similar techniques have become available for studies of local CBF and

CMR_{gl} in man; in these, tracer uptake is assessed by positron emission tomography (see Kuhl et al. 1980).

In spite of the fact that all these techniques, and a variety of others, have been used in the pursuit of factors and mechanisms influencing tissue perfusion, we still lack precise information on how CBF is regulated in health and disease. In order to discuss the basic problems involved, it seems justified to review briefly the main features of CBF regulation.

1. Circulatory homeostasis in the brain

When discussing CBF regulation it is profitable to recall three features of this regulation: (a) the cerebral circulation shows autoregulation (b) under normal conditions, metabolic rate and blood flow are closely coupled, and (c) under some conditions, notably hypoxia, hypoglycemia and hyper/hypocapnia, metabolic rate and blood flow are "uncoupled".

a. Autoregulation. This term describes the observation that when cerebral perfusion pressure is varied within relatively wide limits (60-160 mm Hg), CBF remains constant. The phenomenon was first demonstrated by Fog (1934), using qualitative techniques, and was subsequently documented with quantitative techniques in man (see Lassen 1959) and experimental animals (Rapela and Green 1964, Harper 1965, Häggendal and Johansson 1965). At the lower limit of autoregulation, CBF falls, and at the upper limit CBF increases above normal ("breakthrough of autoregulation", see Strandgaard et al. 1974, MacKenzie et al. 1976c).

b. Coupling of metabolic rate and blood flow. It is known that, under normal and some pathological conditions cerebral metabolism and blood flow are closely coupled i.e. there exists a direct relationship between CBF and CMR_{O₂} (or CMR_{gl}). The coupling suggests that CBF is proportional to metabolic rate, the latter reflecting the intensity of nervous tissue activity. This holds true not only for physiological variations (e.g. the increase in local CBF upon sensory or motor activation see Olesen 1971) but also for some pathological states involving reduced (e.g. anaesthesia, hypothermia, and metabolic coma) or increased (e.g. hyperthermia and epileptic seizures) cerebral metabolism. The coupling is also evident when local CBF

is related to local CMRgl in any given physiological state (see Sokoloff 1978).

c. Uncoupling of metabolic rate and blood flow. It would seem that the increase in CBF observed in hypoxia and hypoglycemia represents a homeostatic mechanism which serves the purpose of increasing oxygen and substrate delivery to the cells. However, an uncoupling is also observed under conditions of an altered carbon dioxide tension in the arterial blood (PaCO_2). Thus if PaCO_2 is reduced to below half its normal value CBF falls to 50-70% of control, and when PaCO_2 is doubled CBF increases markedly; in the rat, flow rates may reach 400-500% of control. These CBF changes seem less purposeful than those observed in hypoxia and hypoglycemia since they appear to increase oxygen and substrate supply beyond the needs.

2. Possible coupling mechanisms

Throughout the years, interest has mainly been focussed on feedback control by products of an altered metabolism, and on neurotransmitters and neuromodulators. When discussing the latter, we will consider separately mechanisms related to the extrinsic and intrinsic innervation of cerebral vessels, and those representing possible coupling factors with a less obvious relationship to a vascular innervation.

a. Metabolic feedback control. When considering metabolic factors influencing CBF the following facts should be recalled. First, since glucose is usually metabolised to CO_2 and water by brain cells, CO_2 production is proportional to functional activity. Second, at least during intense activity, some of the glucose utilized will be metabolized to lactic acid. Thus, increased functional activity leads to enhanced production of hydrogen ions. Third, functional activity leads to accumulation of K^+ in extracellular fluid, and to loss of Ca^{2+} . Fourth, when ATP is hydrolyzed during activity, conditions are at hand for accumulation of AMP, and for its dephosphorylation to adenosine.

All the consequences of increased activity mentioned (reductions in extracellular pH and Ca^{2+} activity, increases in extracellular K^+ activity and adenosine concentration) have been proposed as coupling factors (Betz 1972, Kuchinsky and

Wahl 1978). However, studies on CBF changes in hypoxia, hypoglycemia, hypercapnia, and epileptic seizures have shown that none of these factors can provide a likely "unified" mechanism of CBF regulation (Astrup et al. 1978, Nilsson et al. 1978, Siesjö et al. 1980).

b. Extrinsic innervation. Early anatomical work showed that the major cerebral arteries are accompanied by autonomic nerves, both sympathetic and parasympathetic (e.g. Stöhr 1922, Forbes 1928). Subsequent histological (Rennels and Nelson 1975) and fluorescence histochemical (see Edvinsson and MacKenzie 1977, Owman and Edvinsson 1977) work showed that this innervation mainly supplies the extraparenchymal arteries (and veins); however, some nerve fibres accompany the smaller arterial branches into the parenchyma.

The sympathetic fibres have their nerve cells localized to cervical ganglia. These fibres provide a noradrenergic innervation, mediating vasoconstriction via alpha-receptors. There is no evidence that this innervation provides a major mechanism of CBF control. Thus, cervical sympathectomy or stimulation of the cervical sympathetic nerves has but small effects on CBF. Evidence exists, though, that manipulation of the extrinsic sympathetic innervation shifts the autoregulatory curve along the pressure axis. Thus, whereas stimulation of the cervical sympathetic nerves extends the autoregulation to higher pressures (Bill and Linder 1976) sectioning of the nerves allows the pressure to fall further without a reduction in CBF (Fitch et al. 1975, Harper et al. 1977). However, it should be emphasized that the extrinsic noradrenergic system is not responsible for autoregulation. For example, it has been shown that patients with high cervical cord transection retain a capacity for autoregulation (Nanda et al. 1976).

An attractive explanation of the normally small influence of a relatively dense innervation is the series resistance theory proposed by Harper et al. (1972). According to this theory, constriction or dilatation of innervated proximal arteries are counteracted by inverse changes in more distant vessels which are under metabolic control. Thus clear effects of, for example, sympathetic stimulation are only observed if the distal vessels

are dilated by for example hypercapnia.

Less is known about the corresponding parasympathetic innervation, which is cholinergic, mediating vasodilatation. In general, though, surgical or pharmacological intervention with this system has only moderate effects on tissue perfusion (Kuschinsky et al. 1974, Vasques and Purves 1977).

c. Intrinsic innervation. Recent results support the existence of an intrinsic innervation of brain vessels, i.e. one that has its origin within the brain itself. Current interest focuses on monoaminergic neuron systems, that have their cell bodies localized to discrete nuclei in the brain stem (Lindvall and Björklund 1978), and particularly on the noradrenergic system that originates in the nucleus locus coeruleus (see Amaral and Sinnamon 1977). When stimulated, this system exerts an inhibitory effect upon nerve cells in the cerebellum, hippocampus, and cerebral cortex, probably due to hyperpolarization mediated by beta-receptors (see Hoffer et al. 1973, Segal and Bloom 1976a,b, Phillis and Kostopoulos 1977). At the capillary level, adrenergic nerve fibres have been demonstrated to terminate on capillary pericytes and endothelial cells (Rennels and Nelson 1975, Swanson et al. 1977). It has been suggested that this "innervation" exerts a resting cerebrovascular tone and influences brain capillary permeability (Raichle et al. 1975, Bates et al. 1977). This constrictory tone was reported to be mediated by alpha-receptors since intraventricular administration of phentolamine caused an increase in CBF (Raichle et al. 1975). However, as we will discuss below such an influence is far from established. Besides, the hypothesis fails to take into account the localization of beta-receptors to cerebral microvessels (Herbst et al. 1979, Nathanson and Glaser 1979).

At present, evidence exists that changes in CBF can be induced by modulations of central dopaminergic and serotonergic neurons (see below), and evidence for serotonergic innervation of cerebral microvessels has been presented (Reinhard et al. 1979). However, the changes in CBF may be secondary to changes in metabolism.

d. Circulating amines. At first sight, it is tempting to assume that administration of monoamines could give insight into the

cerebrovascular (and metabolic) effects of intrinsic aminergic systems. However, monoamines are normally unable to pass from blood to brain tissue due to the presence of the blood-brain barrier. For monoamines, this has two components: tight junctions between the vascular endothelial cells constituting a mechanical barrier, and the presence of monoamine inactivating enzymes in the vessel walls, constituting a chemical barrier (see Hardebo and Owman 1980). Hence, it is necessary to bypass the barrier, e.g. by intraventricular administration, or to disrupt it by osmotic or mechanical means (e.g. by raising the blood pressure). By such experiments, it has been shown that noradrenaline, adrenaline, and dopamine agonist (apomorphine) induce increases in CBF, CMR_{O_2} , and CMR_{gl} (MacKenzie et al. 1976a,b, Berntman et al. 1978b, Dahlgren et al. 1980, McCulloch and Harper 1977) while serotonin has opposite effects (Harper and MacKenzie 1977).

e. Other neuromodulators. Evidence is accumulating that other neurohormones/neuromodulators, e.g. peptides, have vascular effects in the brain (Owman and Edvinsson 1977). However, most of the evidence concerns prostaglandins. As described by Vane, Moncada and collaborators, some vascular beds have a dual prostaglandin system in which prostacyclin synthesized in the vessel walls mediates dilatation and thromboxane A_2 , synthesized in platelets, mediates vasoconstriction (see Moncada and Vane 1978). Brain tissues have the capacity of synthesizing prostaglandins (Wolfe et al. 1976a,b, Abdel-Halim et al. 1978). A role for prostaglandins in regulating CBF was first suggested by results published by Pickard and MacKenzie (1973) who found that indomethacin, a potent inhibitor of prostaglandin synthesis (Flower 1974), reduced resting CBF by almost 40%. We will return to this issue below.

3. Hypercapnia and regulation of CBF

In pursuing factors regulating CBF we chose to study hypercapnia, and particularly the modulating influence of intrinsic monoaminergic systems and of prostaglandin synthesis inhibition upon the CO_2 responsiveness of the cerebral circulation, for the following reasons. First, hypercapnia dissociates CBF and CMR_{O_2} without interfering with oxygen or substrate supply to

the tissue. Second, an increase in CO_2 tension mimics conditions during cell activation in the sense that it causes accumulation of one major end product of metabolism. Third, hypercapnia has been shown to increase the rate of hydroxylation of tyrosine and tryptophan in the brain, and to enhance noradrenaline turnover (Carlsson A. et al. 1977, Prous et al. 1977). Fourth, results were at hand showing that measures that affect turnover of noradrenaline in the tissue (e.g. administration of diazepam, see Berntman et al. 1979a) or decrease prostaglandin synthesis (administration of indomethacin, see Pickard and MacKenzie 1973) have pronounced effects on the CBF response to hypercapnia. Following a brief summary of cerebral metabolic changes induced by hypercapnia we will consider available evidence that the neurotransmitter/neuromodulator systems discussed influence the circulatory and metabolic response to hypercapnia.

a. Hypercapnia and brain metabolism

The statement that hypercapnia uncouples CBF and CMR_{O_2} and does not curtail oxygen and glucose supply is an over-simplification and requires modification. Hypercapnia gives rise to alterations in nervous tissue metabolism and function that must be taken into account (see Woodbury and Karler 1960, Siesjö 1978, chapter 10). For example, hypercapnia alters intermediary metabolism with a reduced glycolytic rate through an inhibition of the phosphofructokinase step. Secondary to this, one observes diminished pools of citric acid cycle intermediates and associated amino acids; however, the energy state of the tissue is unchanged.

A much debated question is whether hypercapnia affects CMR_{O_2} or not (see Siesjö 1980). Until a few years ago, it was commonly held that CMR_{O_2} remains constant during hypercapnia (see Smith and Wollman 1972). However, Berntman et al. (1979a) and Hemmingsen et al. (1979), both groups working on rats, reported that hypercapnia significantly increases CMR_{O_2} (by 25% and 35%, respectively). Other reports have been published suggesting that hypercapnia causes no change in CMR_{O_2} , or actually decreases it (Artru and Michenfelder 1980, Kliefoth et al. 1979). These results were obtained in dogs and baboons, respec-

tively. It is tempting to conclude that the differences in results could be explained by differences in techniques, or in species. However, the results reported in rats were obtained with different techniques. Furthermore, Pickard and MacKenzie (1973) obtained results contrary to those of Kliefoth et al. (1979) although the same species was studied. It seemed necessary, therefore, to pay attention not only to changes in CBF but also in CMR_{O_2} . In this context, it should be emphasized that since hypercapnia inhibits glycolysis and leads to consumption of endogenous substrates (see above) measurements of CMR_{gl} do not give valid estimates of metabolic rate.

As already remarked, hypercapnia increases synthesis rates of catechol- and indoleamines in the brain. The results obtained suggest that activity in central noradrenergic and serotonergic neurons is enhanced while the activity of dopaminergic neurons is depressed (Prous et al. 1977). However, hypercapnia is also accompanied by an increased liberation of catecholamines from sympathetic nerve endings and the adrenal glands (Tenney 1956, Morris and Miller 1962, Nahas et al. 1967, Nahas and Steinsland 1968). The question must be asked, therefore, if circulating catecholamines influence the cerebral circulatory and metabolic response to hypercapnia, especially since hypercapnia is known to induce dysfunction of the blood-brain barrier (see Johansson and Nilsson 1977).

b. Influence of intrinsic monoaminergic systems

Manipulations of the extrinsic sympathetic innervation of brain vessels have little or no effects on the CBF response to hypercapnia (Eklöf et al. 1971, Harper et al. 1972, Hernandez et al. 1977, see also Edvinsson and MacKenzie 1976). Thus, the only established effect is a moderate decrease in CBF during hypercapnia by sympathetic nerve stimulation or administration of alpha-receptor agonists (Harper et al. 1972), and there is no indication that the system influences metabolism. Since equally small effects are induced by the extrinsic parasympathetic system (Hoff et al. 1975, Scremin et al. 1977) we will concentrate the discussion to the intrinsic monoaminergic systems. Early studies showed a curtailed cerebrovascular response to hypercapnia following pontine lesions (Shalit et

al. 1967). However, these studies gave no hints as to the nature of the cell systems affected. As stated, two studies suggest that the noradrenergic locus coeruleus system exerts an inhibitory tone on cerebral vessels (Raichle et al. 1975, Bates et al. 1977, see also Sharp and Schwartz 1977). One group reported that destruction of the locus coeruleus system in cats dramatically curtailed the CO₂ responsiveness (Bates et al. 1977), and another that intracisternal injection of 6-hydroxy-dopamine, a neurotoxin that destroys catecholaminergic neurons, had a similar effect (Mendelow et al. 1977). However, Edvinsson et al. (1977) found an increased cerebrovascular reactivity after intraventricular administration of this substance.

Several groups have studied the effects of propranolol, a selective blocker of beta-adrenoceptors. When these studies are considered it should be held in mind that beta-receptors are found not only in cerebral microvessels (see above) but also in nerve and glial cells. The possibility remains, therefore, that any effect of propranolol on CBF is secondary to blockade of cellular receptors. Several reports demonstrate an inhibitory cerebrovascular effect of propranolol during hypercapnia (Aoyagi et al. 1976, MacKenzie et al. 1976b, Berntman et al. 1979a, Hemmingsen et al. 1979, see also Savaki et al. 1978) and it cannot be excluded that the reduction in CBF was entirely caused by metabolic depression. However, the results are equivocal since at least one group has failed to find an effect of propranolol on the CBF response to hypercapnia (Olesen et al. 1978).

So far, dopaminergic neurons have not been shown to make "synaptic" contact with vascular structures; however, in the striatum, dopaminergic fibres run in close association with microvessels (O.Lindvall, pers.comm.). As already stated, dopamine agonists have been found to increase CBF and CMRO₂ (McCulloch and Harper 1977), and these increases were prevented by pimozide, a dopamine receptor blocking substance. However, pimozide did not change "resting" CBF and CMRO₂ and the cerebro-vascular response to hypercapnia was preserved.

Exogenous serotonin, when administered after disruption of the blood-brain barrier, reduces CBF and CMRO₂ (Harper and MacKenzie 1977). This agrees with the general assumption that

the serotonergic system is inhibitory. Analyses of serotonin in the microvascular fraction of cortical homogenates have provided information suggesting the presence of serotonergic innervation in brain microvessels (Reinhard et al. 1979). At present, the only indication that the serotonergic system can influence CO₂ responsiveness is the study of Deshmukh and Harper (1973). In that, a reduction in hypercapnic CBF was noted following intracarotid infusion of serotonin (1 µg·kg⁻¹·min⁻¹).

Until quite recently, the most pronounced effect on the CO₂ responsiveness of the cerebral circulation was that due to diazepam, a drug that reduced hypercapnic CBF to 35% of "control" with an associated reduction in CMRO₂ (Berntman et al. 1979a). However, although diazepam prevents a stress-induced increase in noradrenaline turnover in the brain (Taylor and Laverty 1969, Corrodi et al. 1971) it influences serotonin turnover as well (Dominic et al. 1975), and the drug has such diverse effects that it is impossible to interpret the results as being secondary to inhibition of monoamine turnover.

c. Influence of indomethacin

Pickard and MacKenzie (1973) demonstrated that indomethacin, a blocker of fatty acid cyclooxygenase and thereby of prostaglandin synthesis, had by far the most pronounced effect on the CO₂ responsiveness of the cerebral circulation hitherto observed. These results were confirmed in experiments on rats by Sakabe and Siesjö (1979). These authors also showed that although indomethacin dramatically curtailed the circulatory response to hypercapnia, it had no effect on the response to hypoxia. Subsequently, it could be shown that the drug also failed to reduce the hyperemia associated with hypoglycemia (Nilsson et al. 1981) or with epileptic seizures (Ingvar et al. 1981). Considered together with the finding that, following the administration of indomethacin, the brain retains its capacity of autoregulation (Pickard et al. 1977), it is clear that the drug only affects resting CBF and CO₂ responsiveness.

Although the results obtained in baboons and rats seem clear-cut, contrasting data have been reported for rabbits (Cuypers et al. 1978) and cats (Wei et al. 1980) under barbiturate

anaesthesia. These results raise the question whether species differences are involved or whether barbiturate anaesthesia can blunt the effect of indomethacin.

OBJECTIVES OF PRESENT STUDY

With the background information summarized we set out to evaluate the influence of different mechanisms putatively involved in maintaining normal cerebrovascular tone, and the vasomotor response elicited by hypercapnia.

In the first part of the study, emphasis was laid on the role played by the intrinsic aminergic neuron systems of the brain. To that end, measures were instituted to lesion or block these systems. However, studies were also made to help clarify the role of circulating catecholamines, and of beta-receptor blockade.

In the second part of the study, the modulating effects of prostaglandins on CO₂ responsiveness were evaluated, based on experiments in which prostaglandin synthesis was inhibited by indomethacin.

METHODS

1. Animals, operative and sampling techniques

a. General. All experiments were performed on male Wistar rats, about 3 months of age (SPF strain, Møllegaards Avlslaboratorium, Copenhagen, Denmark). The animals were allowed pellet food and tap water until operation. Experiments on anaesthetized animals were performed during daytime, while those on awake animals were performed in the evening to reduce ambient noise.

b. Preparation of anaesthetized animals. Except for animals anaesthetized with barbiturates and ventilated with N₂/O₂ (70:30) all preparations were performed under Halothane - N₂O/O₂ (70:30) anaesthesia. Immobilizations was accomplished

by d-tubocurarine and ventilation was performed through a tracheostomy with a Starling type respirator. Operative procedures included cannulation of femoral and/or brachial arteries (for measurements of blood pressure, pH, blood gases, oxygen and glucose contents, and arterial tracer concentrations), and one or both femoral veins (for injection of drugs, tracer, and donor blood), as well as exposure of the superior sagittal sinus (for sampling of cerebrovenous blood). In many experiments, electrodes were inserted in the skull bone for recording of the EEG. A thermistor was inserted in the rectum for measurements of body temperature. After the preparation the halothane supply was discontinued and sufficient time for its elimination allowed before further procedures were undertaken. In all experiments heparin was used as anticoagulant.

c. Induction of hypercapnia. Hypercapnia was instituted by substituting 5-6% of the N_2O or N_2 in the inhaled gas mixture for CO_2 . This gave a $PaCO_2$ of about 75 mm Hg.

d. Induction of hypoxia. Hypoxia was instituted by substituting about 20% of the oxygen in the inhaled gas mixture for N_2 . This gave a PaO_2 of about 25 mm Hg.

e. Stereotactical lesions. Stereotactical lesions and appropriate shamlesions were performed with the rat under barbiturate anaesthesia. The animal was fixed in a stereotactic instrument (Kopf Instrument Co., Tujunga, Calif. USA). Experimental proceedings were performed 1 to 31 days after the lesioning as specified for each study.

f. Drugs. The long acting local anaesthetic bupivacaine ($2.5 \text{ mg}\cdot\text{ml}^{-1}$), d-tubocurarine ($3 \text{ mg}\cdot\text{ml}^{-1}$), phenobarbital ($200 \text{ mg}\cdot\text{ml}^{-1}$), and propranolol ($1.0 \text{ mg}\cdot\text{ml}^{-1}$) were used in stock solutions as provided. Heparin was diluted by Krebs-Hensleits solution to contain $300 \text{ I.U.}\cdot\text{ml}^{-1}$. Indomethacin (Confortid^(R), Dumex, Helsingborg, Sweden) was dissolved in distilled water to give a concentration of $10 \text{ mg}\cdot\text{ml}^{-1}$. ^{14}C -hexadecane (standard solution) and ^{14}C -iodoantipyrine was provided by Radiochemical Center, Amersham, England. The ^{14}C -iodoantipyrine was dissolved in ethanol to give an activity of $100 \text{ }\mu\text{Ci}\cdot\text{ml}^{-1}$. The ethanol was evaporated by N_2 and the substance dissolved in Krebs-

Hensleit solution just prior to each experiment.

2. Special procedures and techniques

a. Techniques for measuring CBF. Cerebral blood flow was determined with three different techniques.

The Kety-Schmidt technique: Regional cerebral blood flow was determined with a modification of the Kety-Schmidt technique, where ^{133}Xe was used as diffusible tracer, with sampling of cerebrovenous blood during the desaturation period (Norberg and Siesjö 1974, Berntman et al. 1979b). A partition coefficient for ^{133}Xe of 0.83 was used. In all studies but one (I), arterial blood was collected from a catheter inserted in the brachial artery. This preparation allows a more accurate determination of the cerebro-arterial ^{133}Xe content with respect to time (for details, see Paper III). Constancy in CBF during desaturation was controlled through repeated determinations of arteriovenous differences in total oxygen content (AVDO_2). Arterial and venous blood were simultaneously collected, 2 samples just prior to and 2 during the desaturation process. A spread in values not exceeding 15% was allowed. CMR_{O_2} was calculated by multiplying the CBF-value obtained by a mean of at least three AVDO_2 estimations.

The autoradiographic technique: Local cerebral blood flow (l-CBF) was determined according to Sakurada et al. (1978) with ^{14}C -iodoantipyrine as diffusible tracer. Under normal and low flow conditions 50 μCi of ^{14}C -iodoantipyrine was infused i.v. during 45 sec. For high flow conditions 30 μCi of tracer substance was infused i.v. during 20 sec. Repeated sampling of arterial blood for determination of ^{14}C -activity was performed through a catheter inserted in the brachial artery (for further details, see Paper III). Arterial ^{14}C -activity was determined by liquid scintillation (Rackbeta 1215, LKB Wallac, Turku, Finland). Efficiency in counting was controlled with ^{14}C -hexadecane as internal standard. Tissue activity was measured autoradiographically with a densitometer having an aperture of 1 mm^2 (MacBeth TD 501, Newburgh, N.Y., USA).

The venous outflow technique: Global CBF was measured with a venous outflow technique (Nilsson 1974, Nilsson and Siesjö 1980). This method was used for studying dynamic changes in

CBF, and dose-response curves following administration of indomethacin (Paper V).

b. Analyses of blood gases, pH and total oxygen content in blood. Arterial blood gas tensions and pH were measured anaerobically with microelectrodes (Eschweiler and Co., Kiel, W. Germany, and Radiometer, Copenhagen, Denmark). Values were temperature corrected. Total oxygen content in arterial and cerebrovenous blood was measured polarographically according to Borgström et al. (1974).

c. Paravertebral blockade. Blockade of the lower part of the paravertebral chains was performed percutaneously on anaesthetized rats, with bupivacaine ($2.5 \text{ mg} \cdot \text{ml}^{-1}$, Marcain^(R), Astra, Södertälje, Sweden). When performing this blockade the local anaesthetic solution was coloured by Evans blue thus making it possible to check the localization and spread of the injectate. In paralyzed animals a decrease in mean arterial blood pressure of at least 30 mm Hg within 5 min was considered as a sign of adequate blockade. If this was not accomplished after two injections of 0.1 ml of local anaesthetic on each side the animal was discarded. In experiments on awake animals given paravertebral blockade (PVB) the efficacy of the blocking procedure was assessed by the localization of the injectate. All animals were examined post-mortem to determine the localization and spread of the injectate. If the procedure caused gross bleeding intra- or retroperitoneally or intrapleurally the animal was discarded, as were animals with signs of correct deposition of local anaesthetic on one side only, or with blue discolouration of the medulla. Arterial blood concentration of bupivacaine, 45 min after injection, was determined by mass-fragmentography (Caldwell et al. 1977), kindly performed by Astra Pharmaceutical Co., Södertälje, Sweden.

d. Preparation for experiments on awake animals. During halothane (1%) $\text{N}_2\text{O}/\text{O}_2$ (70:30) anaesthesia delivered by a face mask catheters were inserted proximally in a tail artery and vein. About 0.1 ml of bupivacaine was injected paravasally and at the incision sites, which were then carefully sutured. A thermistor probe (Ellab, Copenhagen, Denmark) was inserted into the des-

ceding part of the colon. The probe was fixed by a suture to the tail. After this the face mask was taken away and, still under anaesthesia, the animal was placed in a Perspex cage. The cage was cylindrical with a flat bottom and its size could be adapted to fit the length of the animal by means of a movable rear end through which the tail with catheters and thermistor probe protruded. The diameter of the cylinder allowed the animal to move its extremities freely but restricted him from turning (Fig. 1). This positioning made it possible to perform rapid decapitation of the rat. In order to shield the animal from its surroundings, aluminium foil was wrapped around

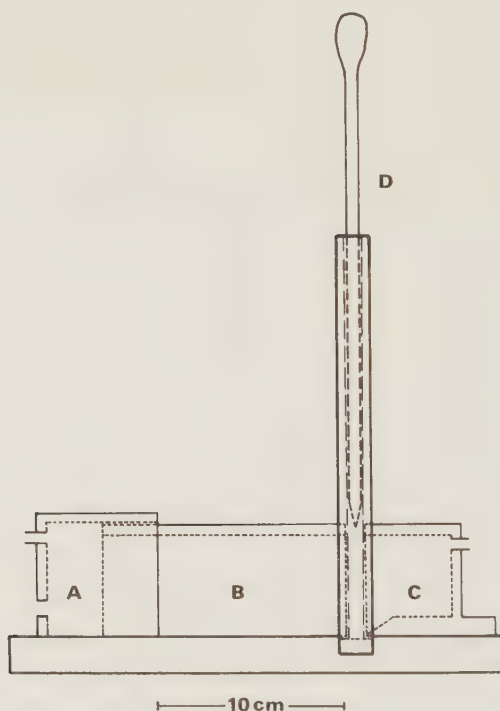


Fig. 1. Perspex cage, side view. A: movable rear end with hole for tail and gas outlet. B: Fixed flat bottomed cylinder. C: Removable front end with gas inlet. D: guillotine axe. For further description, see text.

the cylinder. Through a small opening in the wrapping the animal's head could be inspected. The cage was flushed with air at a rate of $1.5 \text{ l} \cdot \text{min}^{-1}$ and 30 min was allowed for elimination of the anaesthetic gases. This time was followed by a 45 min experimental period, preceeding l-CBF determination as described above.

During the experiment the animal was continuously observed with registration of blood pressure, temperature, and repeated determinations of arterial blood gases, pH, and blood glucose concentration (determined reflectometrically). Any animal showing signs of stress (vigorous movements, rapid changes in blood pressure, a marked decrease in PaCO_2 or increase in blood glucose concentration) during the last 10 min of the experimental period was excluded.

Calculations: Local CBF was calculated using the equation given by Sakurada et al. (1978). Statistical calculations of significance in differences between groups were performed using the Student's t-test for unpaired data or the Mann-Whitney U-test as specified.

RESULTS AND DISCUSSION

Paper I.

The study was designed with two objectives in mind. In the first part of the study experiments were performed in order to investigate whether the noradrenergic locus coeruleus system exerts any resting tone upon CBF and CMR_{O_2} . In the second part of the study the influence of the ascending catecholaminergic bundles upon the cerebrovascular (and metabolic) responses to hypercapnia and hypoxia were studied. Cerebral blood flow and CMR_{O_2} was measured using the Kety-Schmidt technique in all experiments. The extent and effects of lesioning were verified histologically and by means of fluorescence histology.

1. Does the locus coeruleus exert any resting tone upon CBF and CMR_{O_2} ?

a. Effect of lesioning: In the first part of the study animals

with bilateral electrothermic lesioning of the locus coeruleus were studied after 9 to 31 days (most of them after 14 days) under normocapnic conditions. The results were compared to a group comprising three different types of shamlesioned animals (electrothermic lesioning close to, but not affecting the locus coeruleus; identical procedures but no electrothermic lesioning; animals subjected to craniotomy and opening of the dura) operated upon 14 days prior to the CBF determination.

Destruction of the locus coeruleus was found to eliminate noradrenergic nerve terminals in the cortex, hippocampus, thalamus, cerebellum and brain stem. The noradrenergic innervation in the hypothalamus was intact. The shamlesioning procedures were without any effect upon the catecholaminergic innervation.

b. CBF and CMRO₂

Following the electrothermic lesions, values for CBF and CMRO₂ were increased as compared to nonlesioned controls, but did not differ significantly from the sham operated group. There was a wide scatter in the values although all animals exhibited locus coeruleus destruction to the same extent and also a similar noradrenergic denervation. Physiological parameters were similar. Two animals with very high CBF values (5.53 and 3.95 ml·g⁻¹·min⁻¹) showed a somewhat larger destruction of brain tissue at the bottom of the 4th ventricle. In order to rule out that hypersensitivity effects had influenced the results, animals were also tested 1 and 3 days after lesioning. These animals did not differ from the others tested 9 to 31 days after lesioning.

2. Do the ascending catecholaminergic bundles influence the CBF and CMRO₂ response to hypercapnia and hypoxia?

a. Effect of lesioning: Animals were subjected to injection of 6-hydroxydopamine (8 µg in 4 µl) dilaterally into the dorsal tegmental bundle, i.e. the ascending fibers from the locus coeruleus. The injections were localized to the caudal mesencephalon rostral to the locus coeruleus. Animals treated identically except that no injection was performed through the cannulas served as controls. In these, no effect upon noradrenergic innervation was found. The 6-hydroxydopamine injection caused a noradrenergic denervation in the cortex, hippocampus,

and thalamus. Due to diffusion of the neurolytic agent catecholaminergic projections to the hypothalamus were also affected. The dopaminergic neuron system was found to be intact. In sham lesioned animals no effect upon the noradrenergic innervations was found.

b. CBF and CMR_{O_2}

CBF and CMR_{O_2} were measured 14 days after lesioning. Normocapnic values for CBF and CMR_{O_2} in the lesioned group were not different from those measured in the shamlesioned groups. The values were more consistent than those obtained in the locus coeruleus lesioned animals, indicating higher specificity with this type of lesioning. During hypercapnia and hypoxia, values for CBF and CMR_{O_2} were identical to these measured in nonlesioned and shamlesioned animals.

These results thus fail to demonstrate that the locus coeruleus neurons exert any resting tone upon CBF and CMR_{O_2} . The findings agree well with similar studies in which CBF was measured after lesioning of the ascending noradrenergic pathways (Norberg et al. 1978) or in which metabolic rate (deoxyglucose technique) was measured after unilateral locus coeruleus lesions (Schwartz 1978). The results also agree with those following total noradrenergic destruction by means of 6-hydroxydopamine administration intraventricularly or intracisternally. These procedures do not influence resting CBF and CMR_{O_2} in normocapnia (Edvinsson et al. 1977, Mendelow et al. 1977). However, the results of Bates et al. (1977) contrast with our own. These authors found a significant increase in the CBF after bilateral electrolytic lesioning of the locus coeruleus in the cat. These different effects of lesioning can probably not be explained by species differences. An anatomical explanation is more likely. Thus, in the cat the locus coeruleus is more diffuse when compared to the rat (Amaral and Sinnamon 1977) and it is, therefore, more difficult to lesion selectively. This explanation is supported by results in the present study in which two animals with a more widespread destruction of brain tissue adjacent to the locus coeruleus exhibited high CBF values.

As discussed in the Introduction, both direct and indirect

evidence has been presented supporting the assumptions that the locus coeruleus exerts a resting tone upon CBF and CMR_{O_2} and that it is involved in the cerebrovascular response to hypercapnia. The results of the present study conclusively show that this is not the case, and they also exclude the possibility that the ascending pathways from the locus coeruleus have any influence upon the cerebrovascular or metabolic response to hypoxia.

Paper II.

Also this study had two objectives. In the first part, we explored a possible dopaminergic control of CBF and CMR_{O_2} under normocapnia and hypercapnia. In the second part of the study, any possible circulatory and metabolic influence exerted by the serotonergic neuron system was studied under normocapnia and hypercapnia.

1. The influence of the nigrostriatal dopaminergic bundle upon 1-CBF in normocapnia and hypercapnia.

a. Lesioning technique and verification of efficacy in lesioning: Selective dopaminergic nigrostriatal bundle lesioning was performed unilaterally by means of 6-hydroxydopamine ($8 \mu\text{g}$ in $4 \mu\text{l}$ of saline with ascorbic acid, $0.2 \text{ mg}\cdot\text{ml}^{-1}$, as antioxidant). The injection, performed stereotactically, was localized to the rostromedial aspect of the substantia nigra. The efficacy of the lesioning was controlled after one week by behavioral testing following intraperitoneal injection of metamphphetamine ($5 \text{ mg}\cdot\text{kg}^{-1}$). Only animals exhibiting a rotational behaviour exceeding 8 turns per minute ipsilateral to the lesioning side were accepted. This response to metamphphetamine has been proved to demonstrate correct placement of the lesion and disappearance of dopamine fluorescence, as well as depletion of a least 95% of the dopamine content in the caudate-putamen (Björklund et al. 1980).

b. Local CBF in normocapnia and hypercapnia: About two weeks after lesioning, local cerebral blood flow was measured autoradiographically, under normocapnia and hypercapnia. Under normocapnia, mean 1-CBF values were found to be about 15% lower in several structures (including the caudate-putamen),

on the lesion side when compared to the non-lesioned hemisphere. The differences were, however, not statistically significant. No significant differences in l-CBF values were obtained when lesioned and non-lesioned animals were compared. Physiological parameters were similar for both groups.

During hypercapnia (PaCO_2 about 75 mm Hg) there was an overall, 2-3-fold increase in l-CBF values in the lesioned animals, a response similar to that observed in non-lesioned animals (Dahlgren et al., Paper III). In most structures, l-CBF values were similar on the lesioned and non-lesioned sides. However, l-CBF in the caudate-putamen was 10% higher on the lesion side. This should be compared to the CBF values in normocapnia being about 10% lower on the lesion side. For further evaluation of the response to hypercapnia, the ratio of l-CBF values for lesion and non-lesion hemispheres were calculated for 6 brain structures, with and without an established dopaminergic innervation. This calculation revealed a significant ($p < 0.001$) difference only for the caudate-putamen, for which the ratio changed from 0.91 ± 0.04 (normocapnia) to 1.11 ± 0.02 (hypercapnia). The results thus indicate that the increase in CBF induced by hypercapnia, is more pronounced in the denervated caudate-putamen. In this structure, therefore, dopaminergic nerve terminals seem to exert slight vasoconstriction during hypercapnia. The study shows, however, that the dopaminergic neuron system has no major influence on cerebrovascular control in normocapnia and hypercapnia.

2. The influence of the serotonergic neuron system in the brain upon CBF and CMR_{O_2} in normocapnia and hypercapnia.

a. Lesioning technique and control of lesioning effects: Lesion of the cerebral 5-hydroxytryptamine containing neuron system was accomplished by intraventricular injection of 150 μg (free base) of 5,7-dihydroxytryptamine creatine sulphate, dissolved in 20 μl saline with ascorbic acid ($0.2 \text{ mg} \cdot \text{ml}^{-1}$) as antioxidant. All animals were pretreated with desipramine ($25 \text{ mg} \cdot \text{kg}^{-1}$ i.p.) in order to protect noradrenergic nerve terminals. Lesioned animals were compared to identically treated animals with intraventricular injection of the vehicle only. The effect of lesioning was tested on all animals. Thus tissue contents of

serotonin (5-HT) in cortex and brainstem were determined by means of high performance liquid chromatography with electrochemical detection according to Hansson and Rosengren (1978). Only animals with a cortical depletion of serotonin exceeding 90% were included in the study. This corresponded to depletion of serotonin in the brain stem of about 45%.

b. CBF and CMR_{O2} in normocapnia and hypercapnia: Fourteen days after lesioning, CBF and CMR_{O2} were measured using the Kety-Schmidt technique, under normocapnia and hypercapnia. As compared to nonlesioned controls (Dahlgren et al. 1981, Paper V) the sham lesioned and the 5 HT-lesioned group had higher mean CBF values (1.10 ± 0.11 , 1.79 ± 0.39 , and 1.75 ± 0.19 ml·g⁻¹·min⁻¹, respectively). The differences were not statistically significant. At least in part, the higher mean values could have been due to a higher PaCO₂ in the lesioned groups. However, the 5 HT-lesioned animals showed an increase in CMR_{O2} of about 10% when compared to the sham lesioned group. If the comparison is made to nonlesioned controls the difference is about 25% (3.99 ± 0.28 vs 4.84 ± 0.24 μmol·g⁻¹·min⁻¹, respectively) and statistically significant ($p < 0.05$). To evaluate any time dependence of changes in CBF and CMR_{O2}, some animals were analyzed 3 days after operation. No significant differences could be observed between 3 and 14 day old lesions.

During hypercapnia, no significant differences in CBF and CMR_{O2} between the groups were observed, indicating that the 5-HT system are not involved in the cerebral circulatory and metabolic responses to hypercapnia.

These results, obtained with the Kety-Schmidt technique, should reveal any cerebrovascular and metabolic influence exerted by cortical projections of the serotonergic neuron system in the brain. After lesioning, and in spite of the fact that only 10% of the serotonergic projections were intact, no effect upon cerebral blood flow could be detected under normocapnia or hypercapnia. However, there was a small reduction in CMR_{O2} under normocapnic conditions. In view of the results reported by Harper and MacKenzie (1977), it is tempting to conclude that serotonin normally maintain an inhibitory tone on cerebral metabolism. However, the present study fails to

demonstrate that the serotonergic system modulates the circulatory and/or metabolic response to hypercapnia.

Paper III.

In this study, we wished to evaluate the effects of the β -adrenoreceptor blocking agent propranolol on l-CBF during normocapnia and hypercapnia.

a. Administration of propranolol: Propranolol was administered i.v. to animals under N_2O/O_2 (70:30) anaesthesia in a dose ($2.5 \text{ mg} \cdot \text{kg}^{-1}$) assumed to accomplish blockade of cerebral β -receptor (Nahorski et al. 1975). Local CBF was measured under normocapnic and hypercapnic conditions 30 min after a bolus injection of propranolol. However, in order to rule out any effect of dissociation of propranolol from (vascular) receptors, the same dose of propranolol was continuously infused for 15 min during hypercapnia preceding l-CBF determination.

b. Measurement of l-CBF: In preliminary hypercapnic experiments l-CBF was measured with the conventional ^{14}C -iodoantipyrine technique (Sakurada et al. 1978; for details, see Abdul-Rahman et al. 1979). However the results were inconsistent with excessively high CBF values being obtained in some structures. The technique was, therefore, modified in two ways a) with respect to arterial catheter placement for sampling of blood for ^{14}C -activity determination (brachial instead of femoral artery) and b) with respect to the time of infusion of tracer (20 sec instead of 30 sec). Following these modifications, consistent and presumably, more accurate l-CBF values were obtained.

In normocapnic animals injected with propranolol, l-CBF in 22 different structures was reduced by 5-15%. However, in none of these structures was the reduction significant. During hypercapnia, propranolol given as a single dose 30 min prior to l-CBF determination induced a similar, but non-significant CBF reduction. Since diffusion of propranolol from receptor sites not could be ruled out (Bylund and Snyder 1976), propranolol ($2.5 \text{ mg} \cdot \text{kg}^{-1}$) was infused continuously for 15 min in a separate group of hypercapnic animals. With this approach significant reduction in l-CBF values were found in five

structures (frontal and sensory-motor cortex, ventroposterior nucleus of the thalamus, substantia nigra and superior olive), in most of the remaining structures the mean l-CBF values were lower than in the hypercapnic controls.

The present study demonstrates, therefore, that propranolol in a dose of $2.5 \text{ mg} \cdot \text{kg}^{-1}$ has only small effects on l-CBF in normocapnia. This conclusion agrees with the results of other studies (MacKenzie et al. 1976b, Olesen et al. 1978, Berntman et al. 1978a). We note that the results also agree with those obtained following lesioning of the locus coeruleus system (see Paper I). Obviously, our results obtained with a single dose of propranolol under hypercapnia are at variance with reports in which propranolol was found to curtail the hypercapnic response (Aoyagi et al. 1976, MacKenzie et al. 1976b, Berntman et al. 1979a, Hemmingsen et al. 1979), but they agree with those of Olesen et al. (1978).

Conflicting results have been reported regarding the duration of action of propranolol. A short duration of action can be deduced from studies by Bylund and Snyder (1976) and Olesen et al. (1978), whereas a more prolonged effect is proposed by Hayes and Cooper (1971), Evans et al. (1973), and Elghozi et al. (1979). The continuous infusion of propranolol resulted in a moderate decrease in l-CBF values. The question must be asked whether this decrease reflects blockade of cerebral β -receptors. Several facts speak against a specific β -receptor mediated effect upon CBF. a) In the cerebellum, hippocampus, and cerebral cortex, β -receptors mediate neuronal inhibition (Hoffer et al. 1973, Phillis and Kostopoulos 1977, Segal and Bloom 1976a,b), and it is difficult to see that dysinhibition could give rise to a decrease in CBF. b) Lesioning of the locus coeruleus does not alter the cerebrovascular and metabolic response to CO_2 (see Paper I). c) In experiments where propranolol was found to reduce CBF during hypercapnia, reductions were also observed in CMR_{O_2} (Aoyagi et al. 1976, MacKenzie et al. 1976b, Berntman et al. 1979a, Hemmingsen et al. 1979). d) Large doses of propranolol drastically reduce local glucose consumption (Savaki et al. 1978) even in structures devoid of noradrenergic innervation. We can add to this that the reduction in CBF we have observed showed no relation-

ship to the known density of β -receptors (Alexander et al. 1975), or of noradrenergic structures. Thus the results of the present study, and of those reported previously, hint that a reduction in CBF after the administration of propranolol is due to metabolic effects of the drug, not associated with blockade of β -adrenoceptors, but rather caused by unspecific effects.

Paper IV.

The objective of this study was to evaluate the influence of sympathoadrenal activation, if any, upon the cerebrovascular and metabolic effects accompanying hypercapnia. We argued that if catecholamines released into the circulation during hypercapnia were responsible for e.g. the increase in CMR_{O_2} , they could possibly account for part of the increase in CBF as well. Since adrenalectomy will only prevent the release of catecholamines from the adrenal medulla we devised a procedure (paraventricular blockade of the sympathetic chain) that would curtail the release of noradrenaline from sympathetic nerves as well.

1. Blocking technique and general effects of PVB

Paravertebral blockade (PVB) was performed as described in the Method section, which lists the requirements for considering the procedure successful. An adequate blockade, as judged by a blue discolouration extending from about the 9th thoracic to about the 3rd lumbar vertebra was present in all animals included in the study. As controls, animals with intramuscular (i.m.) injection of local anaesthetic was used.

No toxic side effects of the blockade could be revealed as judged by EEG recordings (both during normocapnia and hypercapnia). During normocapnia, blood glucose and lactate values were significantly reduced in animals with PVB, as measured 15, 30 and 45 min after blocking. The lactate/pyruvate ratio was significantly lowered after 30 ($p < 0.05$) and 45 ($p < 0.01$) min. Analyses of bupivacaine concentration in blood 45 min after blocking showed that PVB resulted in concentrations of about 100-150 $ng \cdot ml^{-1}$. Blood concentrations were higher in control animals than in those with PVB.

2. CBF and CMRO₂ during normocapnia

Physiological parameters were similar in the control and PVB groups except for mean arterial blood pressure (MABP) which was significantly lower in the PVB group (145 ± 3 vs. 116 ± 4 mmHg). CBF and CMRO₂ were measured with the Kety-Schmidt technique. Values for the control group were not significantly different from uninjected animals (see Dahlgren et al. 1981, Paper V). In the PVB group CBF was significantly reduced (1.04 ± 0.09 vs. 0.74 ± 0.04 ml·g⁻¹·min⁻¹, respectively, $p < 0.05$) and the mean CMRO₂ values was decreased by about 10%. However, this change was not significant (4.02 ± 0.19 vs. 3.59 ± 0.13 μmol·g⁻¹·min⁻¹, respectively).

3. l-CBF during normocapnia

Local CBF was measured autoradiographically as described above. In order to explore whether increased bupivacain concentrations per se had any influence on CBF, a comparison was made between uninjected controls (from Dahlgren et al. 1981, Paper V) and animals with local anaesthetic i.m..

Significant differences in l-CBF between these two groups were found in only 2 of 22 structures. These results support the conclusion, drawn above, that bupivacaine per se does not cause major alterations in CBF. Local CBF values in 22 brain structures for animals given PVB showed a generalized and significant reduction when compared to values obtained in animals given local anaesthetic i.m. The reduction varied between 30 and 40%, i.e. it was of similar magnitude as that obtained with the Kety-Schmidt technique.

4. The influence of PVB on the circulatory and metabolic response to hypercapnia

The effects of hypercapnia in animals with PVB were evaluated using both the Kety-Schmidt and the autoradiographic technique. Hypercapnic animals (Dahlgren et al. 1981, Paper V) served as controls for the Kety-Schmidt measurements. Hypercapnic animals presented in Paper III served as controls for the autoradiographic technique. Physiological parameters for all groups were similar except for a significantly lower MABP in animals given PVB.

a. CBF and CMR_{O2}

The CBF value was lower in the PVB group. However the relative increase in CBF, caused by hypercapnia, was about the same for controls and animals given PVB (about 4.5-fold). From this result we conclude that PVB does not affect the CBF response to hypercapnia. Hypercapnia caused a significant increase in CMR_{O2} in both groups of animals ($p < 0.05$ for controls and $p < 0.01$ for PVB groups). However, since the CMR_{O2} did not differ between the groups PVB obviously did not affect the cerebral metabolic response to hypercapnia.

b. Local CBF

Local-CBF values for 22 brain structures showed a generalized and consistent reduction in animals given PVB. The pattern was almost similar to that obtained under normocapnia except for the parietal cortex where PVB caused a more marked reduction in hypercapnia. In other words, the relative increase in CBF was the same in control and PVB animals.

5. Effects of PVB on unparalyzed animals

Local CBF was measured in awake animals with a procedure as described under Materials and Methods. However, the preparation was completed by performing PVB. These animals behaved as unblocked controls (Dahlgren et al. 1981), and the physiological parameters and blood glucose values were almost the same. Local CBF values for 22 brain structures were virtually identical in animals given PVB when compared to unblocked controls. Thus, the reduction in CBF observed in anaesthetized and paralyzed animals was not encountered in awake animals.

In summary, this study fails to show that the sympathoadrenal activation modified the cerebrovascular and metabolic response to hypercapnia. In addition, it demonstrates that paravertebral blockade of the sympathetic chain reduces overall and local CBF in paralyzed animals ventilated on 70% N₂O. The nature of this interaction is not understood.

Paper V

When it seemed clear that neither the intrinsic monoaminergic systems nor circulating catecholamines had any major effect

on the cerebrovascular response to hypercapnia, or could be made responsible for the increase in CMR_{O_2} , we turned our interest to prostaglandins. As described in the Introduction, the approach was based on the findings reported by Pickard and MacKenzie (1973) and Sakabe and Siesjö (1979). They showed that indomethacin in a dose of $10 \text{ mg} \cdot \text{kg}^{-1}$ reduced normocapnic CBF by 38-50%, with no significant effect on CMR_{O_2} , and dramatically curtailed the CO_2 responsiveness. In this study we extended the previous work on changes in CBF and CMR_{O_2} , measured l-CBF, and performed continuous measurements of CBF to provide data on speed of action of indomethacin, as well as on dose-response curves.

1. Physiological parameters and behaviour in spontaneously breathing animals with and without anaesthesia

Animals were prepared with tail arterial and venous catheters under halothane anaesthesia. In 3 animals a strain gauge transducer was applied around the thorax. Thus, it was possible to follow changes in the respiratory pattern during induced hypercapnia. This response was not altered by the administration of indomethacin ($10 \text{ mg} \cdot \text{kg}^{-1}$). In two animals, fully recovered from anaesthesia, the effect of indomethacin ($10 \text{ mg} \cdot \text{kg}^{-1}$) was observed with regard to behaviour, blood pressure, arterial blood gases, and pH. The injection of indomethacin ($10 \text{ mg} \cdot \text{kg}^{-1}$ i.v.) somewhat reduced motility but not the reactions to auditory or tactile stimulation. The administration of indomethacin caused a slight hyperventilation (PaCO_2 decreased about 5 mmHg), however, pH remained unchanged as did other parameters.

2. CBF and CMR_{O_2}

The effects of indomethacin ($10 \text{ mg} \cdot \text{kg}^{-1}$ i.v.) upon CBF and CMR_{O_2} were evaluated using the Kety-Schmidt technique under normocapnia and hypercapnia. In normocapnic animals CBF determination was performed 30 min after the intravenous injection of the substance. In hypercapnic animals two intervals between the administration of indomethacin and measurements were investigated i.e. 30 and 45 min, with hypercapnia maintained for 25-30 min preceeding CBF measurement. In order to investigate the presence of any interaction between indomethacin and N_2O , 5 normocapnic animals were adrenalectomized and treated accord-

ing to Carlsson C. et al. (1977) and N_2O was withdrawn for the last 15 minutes preceding CBF determination. In normocapnic animals CBF was found to be significantly reduced to about 50% of control with no statistically significant reduction in CMR_{O_2} . No signs of interaction between indomethacin and N_2O could be observed. In control animals, hypercapnia caused a 5-fold increase in CBF (in accordance to Berntman et al. 1979a); however, in this material no significant increase in CMR_{O_2} was found. Indomethacin dramatically reduced the increase in CBF during hypercapnia. There was no effect on CMR_{O_2} . The relative change in CBF during hypercapnia was similar in controls and indomethacin-treated animals. However, the true vascular responsiveness to CO_2 , as expressed by the $\Delta CBF / \Delta PaCO_2$ ratio, was decreased by 75% in indomethacin-treated animals (0.088 vs. $0.021 \text{ ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1} \cdot \text{mm Hg}^{-1}$).

3. Local CBF

The local cerebrovascular response to indomethacin ($10 \text{ mg} \cdot \text{kg}^{-1}$) was evaluated during normocapnia and hypercapnia using the autoradiographic technique for l-CBF. Local CBF was measured 30-40 min after intravenous injection of the drug. In normocapnic animals, indomethacin caused a generalized reduction in all 20 brain structures investigated. The average reduction was about 50%, thus corroborating the previous results obtained with the Kety-Schmidt technique.

Hypercapnia was induced 15 min after injection of the drug, and was maintained for 25 min prior to l-CBF determination. In the indomethacin-treated animals a generalized reduction was found in l-CBF values in all 20 brain structures analyzed. The relative change in l-CBF caused by indomethacin during normocapnia and hypercapnia was about the same (see above).

4. Time course of CBF response

Using the continuous cerebral venous outflow technique according to Nilsson (1974) the time course in CBF reduction following indomethacin administration ($10 \text{ mg} \cdot \text{kg}^{-1}$) was determined during hypercapnia. A reduction in CBF was observed 15 sec after administration of the drug, and the response reached near maximal values between 1 and 2 min after injection. This rapid onset of action indicates that indomethacin exerts its

effect in a compartment in rapid equilibrium with the blood.

5. Dose-response

Utilizing the same venous outflow technique as above we subjected hypercapnic animals to i.v. bolus injections of indomethacin at 3 min intervals. A reduction in CBF was recorded after $1 \text{ mg} \cdot \text{kg}^{-1}$ and maximal effect was obtained after an accumulated dose of $3\text{--}5 \text{ mg} \cdot \text{kg}^{-1}$. This dose response effect was shown to be in perfect agreement with comparable results presented by Abdel-Halim et al. (1978) who studied the effect of indomethacin on the synthesis of brain tissue prostaglandins.

In summary the present study demonstrates a generalized reduction in CBF after the administration of indomethacin ($10 \text{ mg} \cdot \text{kg}^{-1}$). These results confirm previous reports (Pickard and MacKenzie 1973, Sakabe and Siesjö 1979). At normal blood pressure, a comparable reduction in CBF has only been observed in pronounced hypocapnia (e.g. Eklöf et al. 1973). The uncoupling of CBF and CMR_{O_2} in indomethacin-treated animals suggests that the drug primarily inhibits prostacyclin synthesis in the vessel walls (see Pickard et al. 1980). Undoubtedly, prostaglandin-like substances must be considered as putative coupling factors with regard to the cerebrovascular response to CO_2 . The maintained ventilatory response to CO_2 after i.v. injection of indomethacin demonstrates that mechanisms regulating ventilation and the cerebrovascular response to hypercapnia are separable entities.

Paper VI

As remarked in the Introduction, two studies performed in barbiturate-anesthetized animals indicated that in the species used (rabbit and cat) indomethacin neither reduced resting CBF nor curtailed the CO_2 responsiveness. We investigated, therefore, the effects of indomethacin on CBF and CMR_{O_2} under normocapnic and hypercapnic conditions in barbiturate-anaesthetized rats.

Animals were anaesthetized with phenobarbital $150 \text{ mg} \cdot \text{kg}^{-1}$ i.p., paralyzed and ventilated with N_2/O_2 (70:30) and prepared as stated in Materials and Methods. Four groups of animals were studied: normocapnic with and without indomethacin and

hypercapnic with and without indomethacin. CBF and CMR_{O_2} was measured using the Kety-Schmidt technique 45 min after i.v. injection of indomethacin ($10 \text{ mg} \cdot \text{kg}^{-1}$). Hypercapnia was induced 30 min prior to CBF determination.

Normocapnic animals without indomethacin showed expected values for CBF and CMR_{O_2} ($0.55 \pm 0.02 \text{ ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$ and $2.75 \mu\text{mol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$, respectively). The administration of indomethacin to normocapnic animals caused a 20% reduction in CBF ($p < 0.01$). CMR_{O_2} was unchanged.

In hypercapnic animals without indomethacin CBF was $1.97 \pm 0.20 \text{ ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$ and CMR_{O_2} $3.03 \pm 0.15 \mu\text{mol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$. In indomethacin-treated animals CBF was $0.73 \pm 0.03 \text{ ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$ and CMR_{O_2} $2.78 \pm 0.17 \mu\text{mol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$. For CBF, this represents an 1.7-fold increase from control. Thus, although indomethacin did not completely obliterate the CO_2 response it clearly attenuated the increase in CBF. This result is therefore in contrast to those reported by Cuypers et al. (1978) and Wei et al. (1980). Again, CMR_{O_2} was unchanged. The reports of Cuypers et al. (1978) and Wei et al. (1980) are based on qualitative techniques (thermoclearance and observations of pial arterial diameter). It is tempting to conclude that these techniques do not measure nutritional flow to the tissue. Thus, the results reported by Bill (1979) in conscious rabbits (a 20% decrease in CBF following indomethacin administration) suggest that species differences are not involved.

Since the CO_2 response bears a relation to the CMR_{O_2} (Fujishima et al. 1971, Sándor et al. 1977) the effect of indomethacin on the cerebrovascular response to CO_2 under different anaesthetic conditions is best evaluated using the $\Delta\text{CBF}/\Delta\text{PaCO}_2$ ratio. By including results from a previous study (Dahlgren et al. 1981, Study V) the following conclusions can be drawn: Phenobarbital anaesthesia reduces the CO_2 response by about 60% when compared to N_2O (70%) anaesthesia. Indomethacin has a similar effect under N_2O (70%) and phenobarbital anaesthesia (4-5-fold). Two further conclusions could be drawn from the pooled material: Indomethacin leads to a moderate (10-15%) reduction in CMR_{O_2} ($p < 0.01$) and hypercapnia gives rise to an increase (10-15%) in CMR_{O_2} ($p < 0.05$). We note that indomethacin failed to prevent the increase in CMR_{O_2} during hypercapnia.

Paper VII

The objective of the present study was to investigate the influence of anaesthetic procedures upon the circulatory effects caused by indomethacin. Animals were prepared for studying l-CBF in the awake, minimally restrained condition as described under Materials and Methods. Results from the present experiments were compared to simultaneously obtained data for similarly treated animals (Dahlgren et al. 1981) not given indomethacin. Fortyfive min prior to l-CBF determination indomethacin ($10 \text{ mg} \cdot \text{kg}^{-1}$) was injected i.v. No adverse reactions to the substance were observed.

In indomethacin-treated animals, l-CBF showed a generalized and highly significant reduction in all 22 brain structures analyzed. The reduction in CBF varied from 25 to 45%. The least pronounced reduction in l-CBF was observed in two cortical structures (parietal and sensory-motor) and the most pronounced in hypothalamus. Cortical structures, though, were not uniformly affected since two of them (frontal and visual) had reductions of less than 30% and one (auditory cortex) a reduction of 40%.

Since the results obtained showed that indomethacin caused a smaller reduction in l-CBF in awake than in paralyzed and anaesthetized animals it is necessary to discuss how the anaesthetic affects l-CBF. As described in another publication (Dahlgren et al. 1981) N_2O anaesthesia and immobilization increases CBF in some structures (striatum and most cortical areas) but reduces CBF in others (cerebellum, inferior colliculus, superior olive, septal nuclei, hippocampus, amygdala and the hypothalamus).

In order to evaluate the influence of the anaesthetic procedures upon the CBF effects of indomethacin we compared the relative reduction in l-CBF in awake and immobilized (70% N_2O) animals. This comparison allows the following conclusions to be drawn. First, in about half of the 22 brain structures investigated indomethacin caused a similar reduction of l-CBF in the two groups. Second, in nine structures indomethacin caused significantly more pronounced reductions in l-CBF in N_2O anaesthetized animals than in awake animals (cerebral cortical

areas, the striatum, lateral geniculate body, superior colliculus, and the habenula). Third, following administration of indomethacin 1-CBF values in N₂O anaesthetized animals are 10-20% lower than values obtained in awake animals.

Obviously, N₂O and immobilization accentuate the effect of indomethacin, at least in a number of cerebral structures. Taking into account the effects of the anaesthetic procedures per se, we conclude that indomethacin causes a more pronounced reduction in 1-CBF values in structures with an upheld CBF during N₂O anaesthesia, while the effect of indomethacin is less pronounced in regions that show a marked decrease in 1-CBF during anaesthesia.

Conclusions

Based on the results presented the following conclusions can be drawn.

1. The cerebral noradrenergic and dopaminergic neuron systems do not exert any influence upon cerebral perfusion or metabolism under "resting" conditions. The cerebral serotonergic neuron system may have a moderate inhibitory effect upon cerebral metabolism in normocapnia. However, any such influence must be slight since CBF remained unaltered in lesioned animals.

2. The increases in CBF and CMR_{O₂} during hypercapnia are neither affected by lesions of the monoaminergic neuron systems in the brain, nor by measures that curtail an increase of circulating catecholamines.

3. Inhibition of brain prostaglandin synthesis, following the administration of indomethacin, causes a rapid and generalized reduction in CBF. The extent of this reduction is governed by the existing tone in the cerebrovascular structures. This effect on cerebral perfusion is probably caused by an inhibition of prostacyclin synthesis in the cerebral vessels.

Indomethacin reduces the cerebrovascular reactivity to changes in PaCO₂. In all probability, therefore, prostaglandins constitute coupling factors in the CBF response to hypercapnia.

4. Nitrous oxide anaesthesia and immobilization give rise to

circulatory changes within the central nervous system. These changes influence the cerebrovascular response to indomethacin, and also the effect of circulating catecholamines.

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CEREBRAL BLOOD FLOW AND OXYGEN CONSUMPTION IN THE RAT
BRAIN AFTER LESIONS OF THE NORADRENERGIC LOCUS COERULEUS
SYSTEM

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SUMMARY

The effect of lesions of the locus coeruleus neuron system on cerebral metabolic rate for oxygen (CMR_{O_2}) and blood flow (CBF) was evaluated in paralyzed and mechanically ventilated rats, using a ^{133}Xe modification of the Kety-Schmidt inert gas technique. Bilateral electrothermic lesions of the nucleus locus coeruleus or bilateral 6-hydroxydopamine lesions of its ascending bundle caused no significant change in CBF or CMR_{O_2} . The 6-hydroxydopamine lesions did not influence the CBF and CMR_{O_2} responses to hypercapnia and hypoxia.

It is concluded that the locus coeruleus does not exert any resting tone on CBF and CMR_{O_2} and that no influence on the CBF and CMR_{O_2} responses to hypercapnia and hypoxia is mediated via its ascending projections.

INTRODUCTION

The nucleus locus coeruleus gives rise to a diffuse noradrenergic innervation of cells throughout the forebrain, brain stem, cerebellum and spinal cord (for reviews see refs. 2 and 24). It has been generally assumed that the activity of the locus coeruleus neurons is changed in states of altered behavior and mood, and various stressful situations have been shown to be accompanied by an increased noradrenaline turnover^{11,22,48}. Since stimulation of the locus coeruleus has consistently been found to inhibit the firing rates of Purkinje cells in the cerebellum²¹ as well as of hippocampal neurons^{40,41}, it has been widely assumed that noradrenaline predominantly induces hyperpolarization and inhibition of neurons throughout the brain.

Recently, morphological evidence has been presented for a central noradrenergic innervation of microvessels in the brain^{15,19,23,46}. The innervation seems to be localized at the capillary level^{28,37,46}, and it is therefore highly interesting that recent studies suggest the presence of beta-adrenergic but not alpha-adrenergic receptors on capillaries^{18,20,32}. However, other authors have proposed that the locus coeruleus neurons maintain a constrictory alpha-receptor-mediated tone. For example, stimulation of the locus coeruleus has been found to

reduce cerebral blood flow (CBF)^{35,36} and destruction of the nucleus to increase resting CBF, and to curtail the CBF response to induced hypercapnia⁴.

Other results are seemingly discordant with the hypothesis that the intrinsic noradrenergic systems mediate vasoconstriction (and depression of metabolic rate). For example, when noradrenaline is injected intraventricularly, or if its passage from blood to brain is enhanced, both CBF and cerebral metabolic rate for oxygen (CMRO₂) increase^{6,26,27}. Since propranolol abolished these increases, a beta-adrenoreceptor effect is suggested. Other results also demonstrate that drugs that block beta-receptors (propranolol, see ref.27) or prevent a stress-induced increase in noradrenaline turnover (diazepam, see ref. 7) depress CBF and CMRO₂ during hypercapnia. A similar although less pronounced effect of diazepam has been reported in hypoxia⁸.

The objectives of the present study were two-fold. First, we wanted to explore the effects of a destruction of the ascending noradrenergic pathways on CBF and CMRO₂ under normocapnic and normoxic conditions. To achieve this goal, experiments were performed in which the locus coeruleus was lesioned bilaterally by electrocoagulation, or in which the ascending noradrenergic pathways were interrupted by microinjections of 6-hydroxydopamine. Second, we wished to establish whether or not such lesions influence CBF and CMRO₂ under conditions of hypercapnia or hypoxia.

MATERIALS AND METHODS

All experiments were performed on male Wistar rats weighing between 320 and 445 g. The animals had free access to food pellets (San-Bolagen, Malmö, Sweden) and tap water until operation.

Placement and control of lesions

Lesion techniques. All lesions were performed under general anesthesia (Brietal, Lilly, 40 mg/kg i.p.) with the animals placed in a stereotaxic instrument (Kopf Instrument Co., Tujunga, Calif.). Two main types of lesions were made as follows:

Type 1: Lesions of the ascending noradrenaline pathways.

Bilateral transection of the ascending noradrenaline fibers from pons and medulla oblongata was made at the level of the caudal mesencephalon, rostral to the catecholamine-containing cells in the locus coeruleus and subcoeruleus area (Fig. 1). The lesion was produced by injections of 6-hydroxydopamine (8 μg in 4 μl of saline on each side) close to the dorsal tegmental catecholamine bundle. In some animals, which served as controls, the cannula was lowered to the same coordinates but no injection of 6-hydroxydopamine was made.

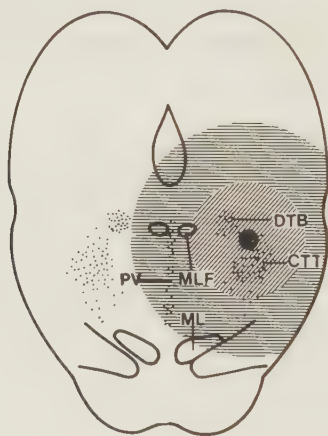


Fig. 1. Position of the bilateral injections of 6-hydroxydopamine in the mesencephalon. The figure illustrates the area of non-specific damage (inner circle), the area of specific lesion of catecholamine fibers (middle circle, estimated on the basis of the observations of Ungerstedt⁴⁹), and the area of diffusion of the injected 6-hydroxydopamine (outer circle, estimated on the basis of distribution of radioactivity after injection of [H^3]dopamine according to Agid et al.¹). Abbreviations: CTT, central tegmental tract; DTB, dorsal tegmental bundle; ML, medial lemniscus; MLF, medial longitudinal fasciculus; PV, paramedian fiber flow.

Type 2: Locus coeruleus lesions. The noradrenaline-containing cell bodies in the locus coeruleus were destroyed bilaterally by electrothermic lesions using a radiofrequency lesion generator (RFG-4, Radionics, Burlington, Mass.). The electrode used had an exposed tip of 0.8 mm. With the tooth-bar at zero the following coordinates were used: 8.5 mm behind bregma, 1 mm lateral, 6.4 mm down from the dura. Lesions were performed by keeping the tip of the electrode at + 56°C for 1 min (Fig. 2). In some control animals (cf. below) the same coordinates were used except that the electrode tip was lowered only 5.4 mm down from the dura (cf. above) and no electrothermic lesion was made.

Fluorescence histochemistry. The brains were perfused with buffer containing a high concentration of magnesium then freeze-dried, according to the method of Lorén et al.²⁵. The extent of the lesions and the denervation effect in cortex, hypothalamus and thalamus were determined.

Measurements of CBF and CMRO₂

Operative and sampling techniques. The animals were anesthetized with 3% halothane and 75% N₂O in oxygen. A tracheostomy was then performed and the animals were paralyzed by D-tubocurarine (1.5 mg/kg) and connected to a Starling type respirator. Ventilation was adjusted to give normoxia (PaO₂ > 100 mmHg) and normocapnia (PaCO₂ 30-40 mmHg). After this procedure the halothane concentration was diminished to 0.7%. The femoral vessels were cannulated to accomplish a line for i.v. injections of drugs and blood, continuous blood pressure recording, anaerobic sampling for blood gas analyses and pH estimation, and blood sampling during CBF measurement. Relevant values were corrected for any deviation of body temperature from 37°C. The bone of the skull was exposed through a small reopening of the skin incision. At this stage, i.e. when the operative procedures were completed, the halothane supply was discontinued. The animals were heparinized (10 IU i.v.) and allowed a steady-state period of at least 30 min.

Induction of hypercapnia-hypoxia. Hypercapnia, PaCO₂ around 80 mm Hg, was induced by addition of CO₂ to the insufflated gas mixture. The period of hypercapnia was 30 min. Hypoxia, PaO₂



Fig.2. Schematic illustration at 5 frontal levels of the position of locus coeruleus lesions (indicated by hatching) in 5 different animals. The drawings, which also show the distribution of catecholamine cell bodies, were modified from Lindvall and Björklund²⁴. Individual values for CBF and CMR_{O₂} are given at the bottom of the figure. Abbreviations: FLM, MLF, fasciculus longitudinalis medialis; G VII, genu of the facial nerve; lc, locus coeruleus; LM, lemniscus medialis; MCP, middle cerebellar peduncle; me V, mesencephalic trigeminal nucleus; ML medial lemniscus; N VII, facial nerve; n V, principal trigeminal nucleus; ns V, spinal trigeminal nucleus; P, pyramidal tract; SCP, superior cerebellar peduncle; soc, superior olivary complex; TS V, spinal tract of the trigeminal nerve; V, trigeminal nerve; V IV, fourth ventricle; vtn, ventral tegmental nucleus.

about 25 mmHg, was induced by reducing oxygen concentration in the gas mixture to which a small amount of CO₂ was added in order to maintain normocapnia. Hypoxia was maintained for 30 min.

CBF-CMR_{O₂} measurement. We measured CBF by means of a modification of the Kety-Schmidt technique using ¹³³Xe as the inert tracer⁸. Prior to and during flow measurement 4 coupled samples of arterial and cerebral venous blood were taken for estimation of arteriovenous differences in oxygen concentration (AVD_{O₂}). The AVD_{O₂} used to derive CMR_{O₂} according to the equation

$$\text{CMR}_{\text{O}_2} = \text{CBF} \cdot \text{AVD}_{\text{O}_2}$$

was the mean of at least three samples. Experiments were omitted if AVD_{O₂} prior to and during flow measurement exceeded 10% in spread.

Statistics

Statistical differences were calculated with the Mann-Whitney U-test.

RESULTS

A. Effects of locus coeruleus lesions

After measurements of CBF and CMR_{O₂} the extent of the lesion and the denervation effects induced by the lesion were analyzed in the fluorescence microscope. Fig. 2 shows the localization of 5 representative locus coeruleus lesions from the present experimental material.

Animals were included in the lesion group only if they had no or only single cells left in the locus coeruleus and if the noradrenergic innervation of the cerebral cortex, hippocampus and cerebellum had been removed. There was also a major denervation in the thalamus but only a minor reduction of noradrenergic fibres in the hypothalamus. The lesions did not affect the dopaminergic neuron systems or the noradrenergic systems originating in pontine and medullary cell groups outside the locus coeruleus (giving rise to the major part of the noradrenergic innervation of the hypothalamus).

The following animals were included in the control group:
(a) 3 animals with lesions localized close to the locus coeruleus in the pons, sparing all the noradrenergic cells in this

nucleus; (b) 3 animals in which the electrode was lowered to a coordinate just above the locus coeruleus and no electrocoagulation was made; and (c) 3 animals in which a craniotomy was performed and the dura was opened as in the other animals but in which the electrode was not lowered into the brain substance. As judged by fluorescence microscopy none of these 'sham lesions' had any effect on the innervation density of the nor-adrenergic and dopaminergic systems. Animals with lesions that affected the locus coeruleus but did not lead to complete and bilateral destruction of the nucleus were not included in the lesion or the sham groups.

The lesion group comprised animals lesioned 9-31 days prior to the CBF and CMR_{O_2} measurements. Most animals were studied 14 days after the lesion but since the 9 and 31 day animals did not differ from the rest they were included in the lesion group. All sham operations were performed 14 days before the experiment. Fig. 3 shows individual CBF and CMR_{O_2} values in sham-

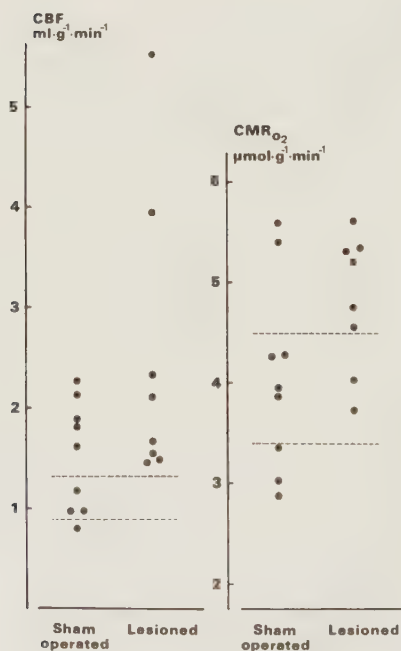


Fig. 3. Individual CBF (left) and CMR_{O_2} (right) values in animals with 'sham lesions' or with bilateral lesions of the locus coeruleus. The interrupted lines demarcate the 95% confidence interval for CBF and CMR_{O_2} , respectively, for the control group.

operated animals and in animals with locus coeruleus lesions. Table I gives the mean values for the groups and also includes the physiological variables. As can be seen, the locus coeruleus-lesioned rats showed increased mean CBF and CMR_{O_2} values. However, these values were not statistically different from those measured in sham operated animals. There was a considerable variation in the CBF values (see Fig.3) and two of the animals had very high values (5.53 and 3.95 ml/g/min, respectively) in spite of the fact that all physiological parameters were similar to those of the rest of the animals. Since all lesions destroyed the locus coeruleus to a similar degree no correlation between the extent of the lesion and the CBF- CMR_{O_2} values obtained was apparent. When comparing the lesions in the two rats with very high CBF values to those of the animals with normal CBF values, we could not attribute the difference to a lesion of any defined structure outside the locus coeruleus. However, the lesions observed in the rats with high CBF values seemed somewhat larger than the others, destroying more brain tissue in the floor of the fourth ventricle (see Fig.2).

Studies on the sympathetic innervation of vessels in the brain (as well as in other organs) have shown that the time after denervation is critical for evaluation of any cerebrovascular parameter (for references and discussion, see ref.17). In order to reveal any time dependent changes in CBF and CMR_{O_2} rats with complete bilateral locus coeruleus lesions were taken for analysis after 1 and 3 days as well. No significant differences in CBF and CMR_{O_2} between rats with lesions of various ages (1-31 days) could be observed.

Table I. Body temperature, mean arterial blood pressure (MABP), arterial blood gases, pH, total oxygen content in arterial blood (Ta_{O_2}), arteriovenous differences for oxygen (AVD_{O_2}), cerebral blood flow (CBF) and metabolic rate for oxygen (CMR_{O_2}) in rats subjected to bilateral electrothermic locus coeruleus lesions or sham operations.

Experimental group	Temp °C	MABP mm Hg	P_{CO_2} mm Hg	P_{O_2} mm Hg	pH	Ta_{O_2} $\mu\text{mol}\cdot\text{ml}^{-1}$	AVD_{O_2} $\mu\text{mol}\cdot\text{ml}^{-1}$	CBF $\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$	CMR_{O_2} $\mu\text{mol}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$
Sham operated n=9	37.0 ±0.1	147 ± 3	37.5 ±0.9	110 ±1	7.38 ±0.01	10.31 ±0.15	2.85 ±0.2	1.52 ±0.11	4.07 ±0.32
Lesioned animals n=8	37.0 ±0.1	148 ± 5	38.9 ±1.2	108 ± 3	7.36 ±0.01	9.98 ±0.45	2.30 ±0.29	2.51 ±0.52	4.83 ±0.24

Effects of lesions of the ascending noradrenergic pathways

In the second part of the study animals with bilateral 6-hydroxydopamine lesions of the ascending bundle from the locus coeruleus (the dorsal tegmental bundle) were used (Fig.1). In these animals the locus coeruleus innervation in the lower brain stem and spinal cord was intact, whereas it was completely removed in the cerebral cortex, thalamus, hypothalamus and hippocampus. Due to spread of 6-hydroxydopamine at the injection site in the caudal mesencephalon the majority of ascending noradrenergic fibers from pontine and medullary cell groups outside the locus coeruleus were also destroyed. Consequently, a marked denervation was also observed in the hypothalamus, the noradrenergic innervation of which originates mainly from these cell groups. The 6-hydroxydopamine injection did not affect the dopaminergic system.

The 6-hydroxydopamine injections and the sham operations were carried out 14 days prior to the CBF and CMR_{O_2} measurements. In the sham-operated animals the injection needle was lowered into the caudal mesencephalon but no 6-hydroxy-dopamine was injected. As shown in Table II, the 6-hydroxydopamine lesions failed to alter CBF or CMR_{O_2} . It should be noted that, in contrast to the CBF- CMR_{O_2} values measured after electrothermic locus coeruleus lesions, those measured in the 6-hydroxydopamine animals showed little scatter (S.E. 0.52 and 0.15 for CBF, respectively).

Table 2. Body temperature, mean arterial blood pressure (MABP), arterial blood gases, pH, total oxygen content in arterial blood (TaO_2), arteriovenous difference for oxygen ($AVDO_2$), cerebral blood flow (CBF) and metabolic rate for oxygen (CMR_{O_2}) in rats with bilateral 6-hydroxydopamine lesions of the ascending noradrenergic fibre bundle from the locus coeruleus at normoxia and normocapnia, hypoxia and hypercapnia.

Experimental group	Temp °C	MABP mm Hg	P_{CO_2} mm Hg	P_{O_2} mm Hg	pH	TaO_2 $\mu\text{mol}\cdot\text{ml}^{-1}$	$AVDO_2$ $\mu\text{mol}\cdot\text{ml}^{-1}$	CBF $\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$	CMR_{O_2} $\mu\text{mol}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$
Sham operated n=4	37.2 ± 0.2	149 ± 5	36.8 ± 1.0	100 ± 3	7.40 ± 0.01	10.51 ± 0.2	2.92 ± 0.43	1.51 ± 0.44	3.81 ± 0.37
Lesioned n=4	37.3 ± 0.2	160 ± 4	39.0 ± 0.7	109 ± 3	7.36 ± 0.01	10.40 ± 0.23	3.08 ± 0.19	1.36 ± 0.15	4.13 ± 0.34
Hypoxia, control n=6	36.6 ± 0.1	122 ± 4	39.4 ± 0.8	26 ± 1	7.18 ± 0.01	2.20 ± 0.07	1.12 ± 0.05	5.69 ± 0.44	6.40 ± 0.28
Lesioned, hypoxia n=4	36.6 ± 0.3	119 ± 7	39.7 ± 1.1	23* ± 1	7.15 ± 0.03	1.99 ± 0.21	1.07 ± 0.07	6.07 ± 0.55	6.64 ± 0.08
Hypercapnia, control n=7	36.6 ± 0.1	156 ± 6	84 ± 2	123 ± 2	7.13 ± 0.01	9.97 ± 0.38	0.82 ± 0.07	5.60 ± 0.55	4.55 ± 0.41
Lesioned, hypercapnia n=6	36.9 ± 0.2	153 ± 4	80 ± 2	123 ± 4	7.15 ± 0.02	9.82 ± 0.22	0.85 ± 0.03	6.17 ± 0.68	5.23 ± 0.53

The 6-hydroxydopamine-lesioned animals were also used to evaluate the possible influence of the locus coeruleus via its ascending bundle on the CBF and CMR_{O_2} responses to hypoxia and hypercapnia. It should be pointed out that since local and descending projections were intact, it was not possible in the present experiments to determine if the locus coeruleus influences the CBF and CMR_{O_2} at hypoxia or hypercapnia via these axonal systems. Table II gives CBF, CMR_{O_2} and physiological variables in control, hypoxic and hypercapnic groups with and without 6-hydroxydopamine lesions. The non-lesioned animals showed the expected CBF and CMR_{O_2} in hypoxia and hypercapnia (cf. refs. 7 and 8). As discussed in ref. 43, the increase in CMR_{O_2} has not been observed in all species. No significant difference was observed between these animals and those with bilateral 6-hydroxydopamine lesions of the ascending noradrenergic pathways.

DISCUSSION

Does the locus coeruleus exert a resting tone on CBF and CMR_{O_2}

One objective of the present study was to find out if the noradrenergic neurons in the locus coeruleus contribute to a 'resting tone' on cerebral metabolism and blood flow. No significant change of CBF or CMR_{O_2} was found after lesions of the locus coeruleus or its ascending bundle. Since no significant difference were found between animals taken for measurements at various time intervals after the lesion (1-31 days), it seems less likely that receptor supersensitivity had any influence on the results (cf. refs. 17 and 45).

The present findings are in good agreement with those of several studies. Thus, similar experiments with uni- and bilateral 6-hydroxydopamine lesions of the ascending noradrenergic pathways showed no significant change in CBF³³. Furthermore, after unilateral 6-hydroxydopamine lesions of the locus coeruleus no change of the regional metabolic rate, as determined by the uptake of deoxyglucose, was noted in the hippocampus and cerebral cortex³⁹. These areas are innervated by terminals originating in the locus coeruleus. Finally more unselective lesions induced by intracisternal³⁰ or intraventricular¹⁶

injections of 6-hydroxydopamine were without effect on CBF and CMR_{O_2} . Such lesions should have removed major parts of the locus coeruleus system, other central catecholamine neuron systems and the sympathetic adrenergic innervation of the brain vessels.

In contrast to the present data and the other studies mentioned, Bates et al.⁴ found a significant increase in resting CBF of cats with bilateral electrolytic lesions of the locus coeruleus. One possible explanation of this discrepancy is that the locus coeruleus has different effects on the resting CBF in various species. It should be emphasized, though, that it is much more difficult to make complete and selective lesion of the locus coeruleus in the cat than in the rat, since the distribution of noradrenergic cells is much more diffuse in the cat². It is noteworthy that Bates et al.⁴ found a marked reduction of noradrenaline in the paraventricular nucleus of the hypothalamus whereas our own lesions gave only a minor denervation in the hypothalamus. The results of Bates et al.⁴ could indicate that some noradrenergic system outside the locus coeruleus, also had been damaged by the lesions, which might contribute to the change in CBF found in their study.

It is, of course, also possible that the lesions of Bates et al.⁴ affected some non-noradrenergic structure situated close to the locus coeruleus and participating in the regulation of CBF. Such an influence could explain the very high blood flow values found in two animals of the present study. It seems highly warranted to study whether increased CBF values in cats are obtained after selective lesions of the locus coeruleus system by 6-hydroxydopamine. The importance of selective neurochemical lesions by 6-hydroxydopamine as a complement to the electrolytic lesions in order to avoid erroneous conclusions about locus coeruleus function has been pointed out by Crawley et al.¹³

Locus coeruleus has been shown to act via a beta-adrenergic receptor on nerve cells in the cerebellum^{21,44}, hippocampus^{40,41} and cerebral cortex³⁴, and it therefore seems reasonable to assume that a resting tone of the locus coeruleus on CMR_{O_2} could be revealed by the administration of a beta-receptor blocker. Such treatment could also disclose a possible tone

on CBF via beta-adrenergic receptors recently demonstrated on brain capillaries (see above). In agreement with the present study in which no such tone was demonstrated, experiments with administration of propranolol to normoxic and normocapnic rats (2.5 and 5.0 mg/kg) performed in our laboratory⁵ showed no effect on CBF and CMR_{O_2} . Other authors have reported a reduction in CMR_{O_2} or deoxyglucose uptake (or CBF) following propranolol treatment^{3,27,31,38}, but this was possibly due to the higher doses of propranolol or the species used. Furthermore, it seems unlikely that changes seen after the administration of beta-blocker are due entirely to the removal of a tone exerted by the locus coeruleus neurons directly on the nerve cells in the various innervation areas. Thus, in the study of Savaki et al.³⁸, propranolol induced a similar decrease in deoxyglucose uptake in the cerebral cortex, which has a dense locus innervation, and in the caudate-putamen in which only scattered noradrenergic terminals are present. In contrast, the dentate gyrus, which is densely innervated by the locus coeruleus, showed no change in deoxyglucose uptake. It should also be noted that there is a poor correlation between the density of beta-receptors and noradrenergic locus coeruleus terminals in various areas^{9,29}.

Does the locus coeruleus exert any influence on cerebral circulatory and metabolic responses to hypercapnia and hypoxia?

The hypothesis of a brain stem center mediating the cerebral circulatory response to hypercapnia is based on the finding that pontine transection or ablation can attenuate or even completely abolish the vasodilation accompanying an increased pCO_2 ⁴². In this context, it is of interest that hypercapnia increases the activity in central noradrenergic neurons¹⁰, and that drugs or surgical manipulations that modulate noradrenergic transmission influence the circulatory and metabolic response to hypercapnia. Diazepam, which is known to prevent an increased activity in noradrenergic neurons during stress^{11,47}, markedly attenuates the CBF and CMR_{O_2} response to increased pCO_2 ⁷. Administration of the beta-adrenergic receptor blocker propranolol also causes a considerable reduction in the CBF response to hypercapnia²⁷ and a reduction of the CMR_{O_2} value

below control⁷. From these studies it seemed possible that a central noradrenergic system, e.g. that originating in the locus coeruleus, is closely connected with or identical to the brain stem center mentioned above. Lesions of the catecholamine systems have given varying results. Whereas Edvinsson et al.²⁴ found a significant increase in CO₂ reactivity after intraventricular 6-hydroxydopamine, Mendelow et al.³⁰, showed that intracisternal 6-hydroxydopamine attenuated the CBF response to hypercapnia with no change in CMRO₂. Bilateral electrolytic lesions of the locus coeruleus markedly reduced the change in CBF in response to hypercapnia⁴. In the present study, 6-hydroxydopamine lesions of the ascending noradrenergic bundle from the locus coeruleus did not change the CBF and CMRO₂ responses to hypercapnia and hypoxia. The results of the present study thus demonstrate that if the locus coeruleus plays a role for the CBF and CMRO₂ changes in hypercapnia and hypoxia, this influence is not mediated via its ascending projection to telencephalic and diencephalic areas.

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Cerebral circulatory response to hypercapnia: Effects of
lesions of central dopaminergic and serotonergic neuron
systems.

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SUMMARY

The present study explores the possibility that the central dopaminergic and serotonergic neuron systems influence CBF under normocapnic and hypercapnic conditions. In the first part of the study, the effect of unilateral 6-hydroxy-dopamine lesion of the nigrostriatal dopamine pathway on local cerebral blood flow (l-CBF) was measured autoradiographically with ^{14}C -iodoantipyrine as the diffusible tracer. The lesion caused no major effect on CBF under normocapnic or hypercapnic conditions. However, the circulatory response to hypercapnia was slightly enhanced (about 10 per cent) in the denervated caudate-putamen. It is suggested that under hypercapnic conditions the dramatic increase in blood flow in the caudate-putamen is normally modulated by a slight vasoconstriction caused by dopamine release from the nigrostriatal system.

In the second part of the study the effect of intraventricular 5,7-dihydroxytryptamine on cerebral metabolic rate for oxygen (CMR_{O_2}) and CBF was evaluated using a ^{133}Xe modification of the Kety-Schmidt inert gas technique. The lesion, which removed about 90 per cent of cortical 5-hydroxy-tryptamine, had no effect on the circulatory response to hypercapnia nor did it alter CMR_{O_2} . Under normocapnic conditions, though, the lesion seemed to induce a slight increase in CMR_{O_2} , which indicates that the serotonergic system exerts a depressant resting tone on metabolic rate in the brain.

INTRODUCTION

Several recent observations suggest that central monoaminergic mechanisms are involved in the well-documented response of the cerebral circulation to changes in arterial CO₂ tension. Hypercapnia has thus been shown to increase the rate of synthesis of catecholamines and 5-hydroxy-tryptamine (5-HT) in the brain⁹. Furthermore, biochemical, morphological and functional evidence has been provided for a central noradrenergic^{23,26,36} and a serotonergic³⁷ innervation of the cerebral microvasculature. Dopaminergic neurons have not, as yet, been shown to terminate on vessels but, in the caudate-putamen, for example, the dopaminergic terminals are closely associated with microvessels. Monoaminergic neurons can, therefore, exert direct influence on the brain microcirculation and since dopamine, noradrenaline and 5-HT are able both to contract and dilate cerebral blood vessels in vitro^{15, 17, 18} it seems likely that changes in the activity of monoaminergic neuron systems during hypercapnia could lead to alterations in blood flow.

Although stimulation of the noradrenergic locus coeruleus system has been reported to reduce cerebral blood flow (CBF)³⁶ experiments with drugs interfering with monoaminergic neurotransmission have suggested that if noradrenergic mechanisms are involved in the vascular response to hypercapnia they mediate vasodilatation. Propranolol markedly attenuates CBF under hypercapnic conditions^{3,31}, which could, at least partly, be explained by its blocking the effects of noradrenaline acting on β -adrenergic receptors localized on the microvessels^{20, 24,33,35}. 5-HT is also able to induce vasodilatation via a β -adrenergic receptor but it seems more likely that increased release of this amine depresses cerebral metabolic rate and possibly also constricts the vessels via specific 5-HT receptors leading to a reduction in CBF^{21,22}. A vasodilatory action of these monoamines in hypercapnia is, however, suggested by the fact that diazepam, which reduces the turnover rate in central noradrenergic⁴³, dopaminergic¹⁰, and serotonergic¹⁴ neurons, curtails the increase in CBF under hypercapnic conditions⁵. It must be pointed out, however, that it is not

known to what extent these propranolol- and diazepam-induced changes in CBF are secondary to alterations in metabolic rate. On the basis of the results described above it seemed highly warranted to study the effects of lesions of intracerebral monoaminergic systems on the CBF response to hypercapnia. In a previous study¹¹ we found no effect of lesions of the noradrenergic locus coeruleus system on CBF under hypercapnic conditions. The objective of the present investigation was to establish if lesions of dopaminergic and serotonergic neuron systems influence the CBF response to hypercapnia.

MATERIALS AND METHODS

A. Animals

All experiments were performed on male Wistar rats weighing between 320 and 445 g. The animals had free access to food pellets (Astra-Ewos, Södertälje, Sweden) and tap water.

B. Lesion techniques

All lesions were performed under general anesthesia (Brietal, Lilly, 40 mg·kg⁻¹, i.p.) with the animals placed in a stereotaxic instrument (Kopf Instrument Co., Tujunga, Calif. USA).

1) Unilateral lesion of the nigrostriatal dopamine system. This was accomplished by injection of 8 µg (free base of 6-hydroxydopamine (6-OHDA, HCl salt; Hässle, Gothenburg, Sweden) in 4 µl of saline during 4 min into the ascending dopamine pathways. Ascorbic acid (0.2 mg·ml⁻¹) was added as antioxidant. The injection coordinates were: 4.5 mm behind bregma 0.9 mm lateral to midline, 8 mm below the dura. The tooth-bar was 3.0 mm below the interaural line. This placement is at the rostromedial aspect of the substantia nigra where the dopamine axons of the nigrostriatal, mesolimbic and mesocortical pathways assemble.

2) Lesion of 5-hydroxytryptamine-containing systems. The rats were given a single injection of 150 µg (free base) of 5,7-dihydroxytryptamine (5,7-DHT) creatinine sulphate (Regis Chemical Co., USA) into the lateral cerebral ventricle. 5,7-DHT was dissolved in sterile saline containing 0.2 mg·ml⁻¹ ascorbic acid; the injection volume was 20 µl. In the sham opera-

ted animals only the vehicle (saline containing ascorbic acid) was given. Forty-five min prior to the 5,7-DHT injection all animals were given the uptake-blocker desipramine ($25 \text{ mg} \cdot \text{kg}^{-1}$, i.p.; Ciba-Geigy, Switzerland) to protect noradrenergic neurons against the neurotoxic action of 5,7-DHT⁶.

C. Control of lesions

1) Behavioural testing. The efficiency of the dopamine bundle lesion was tested one week after the surgical procedure. Met-amphetamine was given ($5 \text{ mg} \cdot \text{kg}^{-1}$, i.p.) and the animals were then tested for amphetamine-induced circling behaviour in a rotometer⁴⁴. Only rats which responded to amphetamine injection with at least $8 \text{ turns} \cdot \text{min}^{-1}$ in the direction of the lesion side were included in the experimental group. In rats run in parallel and analyzed with fluorescence histochemistry according to Lorén et al.³⁰ this rotational behaviour was found to be indicative of correct placement of the lesion and with disappearance of dopamine fluorescence in the ipsilateral caudate-putamen. By chemical analysis, such animals showed more that a 95 per cent reduction of dopamine in the ipsilateral caudate-putamen⁷.

2) 5-hydroxytryptamine assay. Two brain regions were analyzed from each rat: a) The neocortical sample comprised the frontal lobes rostral to the forceps minor and pieces of parietal cortex taken from a 2 mm thick coronal section at the level of the rostral caudate-putamen. b) The brain stem sample contained pons and medulla oblongata from which cerebellum had been removed. After finishing the blood flow measurements, these tissue pieces were immediately dissected out from the brains, wrapped in aluminium foil and frozen in liquid nitrogen. 5-HT levels were then determined using high performance liquid chromatography with electrochemical detection following the procedure described by Hansson and Rosengren¹⁹.

D. Measurements of blood flow and oxygen consumption

1) Operative and sampling techniques. The animals were anesthetized in a jar with a gas mixture containing 3 per cent halothane and 70 per cent nitrous oxide in oxygen and, when unresponsive, they were tracheotomized, paralyzed with d-tubocurarine chloride ($1.5 \text{ mg} \cdot \text{kg}^{-1}$) and connected to a Star-

ling type respirator delivering about 1 percent halothane and 70 per cent N_2O . Body temperature, measured in the rectum, was kept close to $37^{\circ}C$ by means of a heating bulb. Catheters were placed in the femoral arteries for blood pressure recording and sampling of arterial blood, and in one femoral vein for injections and infusion of donor blood. The arterial samples for measurements of local CBF were taken from one brachial artery. For measurements of cortical CBF and CMR_{O_2} the caudal part of the superior sagittal sinus was exposed to allow repeated sampling of cerebral venous blood. At the end of the operative procedures the halothane supply was discontinued. The animals were heparinized ($300 \text{ IU} \cdot \text{kg}^{-1}$, i.v.) and allowed a steady state period of at least 30 min. In some animals hypercapnia (Pa_{CO_2} about 80 mm Hg) was then induced during 30 min by addition of CO_2 to the gas mixture.

2) Methods and analytical techniques. Arterial PCO_2 , PO_2 and pH were measured with microelectrodes. Total oxygen content in arterial and venous blood was measured polarographically⁸. Cortical CBF was measured with a $^{133}\text{xenon}$ modification of the Kety-Schmidt technique^{4, 34}. CMR_{O_2} was calculated from CBF and arteriovenous differences in oxygen concentration (AVD_{O_2}). The AVD_{O_2} used to derive CMR_{O_2} according to the equation

$$CMR_{O_2} = CBF \cdot AVD_{O_2}$$

was the mean of at least three paired samples. Experiments were omitted if deviations in AVD_{O_2} prior to and during flow measurement exceeded 15 per cent. Local CBF was measured with the autoradiographic ^{14}C -iodoantipyrine technique of Sakurada et al.³⁹ following the procedure described earlier¹².

E. Statistics

Statistical differences were calculated by Student's t-test, except for calculation of the significance in differences between flow ratios where the Mann-Whitney U-test was used.

RESULTS

A. Effects of lesions of the nigrostriatal dopaminergic pathway

As observed in the fluorescence histochemical specimens, the lesions of the ascending dopaminergic pathways caused not only a denervation in the caudate-putamen but also a complete, ipsilateral removal of mesencephalic, dopaminergic afferents to several other brain regions, e.g. frontal cortex and amygdala. Since the dopaminergic pathways are uncrossed²⁸, the 6-OHDA lesion caused no denervation on the contralateral side. Local CBF was measured 2 weeks after the lesions had been carried out i.e. 1 week after the behavioural testing with amphetamine. At normocapnia no significant differences in temperature, mean arterial blood pressure, Pa_{O_2} , Pa_{CO_2} , and pH were observed between lesioned and non-lesioned animals¹² (Table 1).

Table 1. Physiological parameters in the experimental groups.

Experimental groups	Temp. °C	MABP mm Hg	Pa_{O_2} mm Hg	Pa_{CO_2} mm Hg	Arterial pH
Normocapnic controls n=6	37.3 ±0.2	144 ±4	103 ±2	38.1 ±1.0	7.42 ±0.02
Normocapnic lesioned n=9	36.9 ±0.1	146 ±4	104 ±4	36.5 ±0.6	7.43 ±0.01
Hypercapnic lesioned n=6	37.1 ±0.1	143 ±3	117 ^x ±1	78.0 ^{xxx} ±1.0	7.15 ^{xxx}

The values are means ± S.E.M. The asterisks indicate significant differences (^x p < 0.05, ^{xxx} p < 0.001) compared to normocapnic lesioned animals.

There was a minor decrease (up to about 15 per cent) in mean CBF in several brain regions on the lesion side (Table 2). These comprised both structures without a known dopaminergic input, e.g. the sensorimotor cortex, and structures where the dopamine innervation had been removed by the lesion, e.g. the caudate-putamen (decrease in mean CBF by about 10 per cent).

Table 2. Local CBF ($\text{ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$) at normocapnia in rats after unilateral 6-hydroxy-dopamine lesion of the nigrostriatal dopamine bundle.

Structures	Non-lesioned (n=6)	6-OHDA-lesioned (n=9)	
		Control side	Lesion side
Frontal cortex	1.58 $\pm$ 0.20	1.40 $\pm$ 0.13	1.25 $\pm$ 0.14
Sensorimotor cortex	1.69 $\pm$ 0.20	1.84 $\pm$ 0.22	1.55 $\pm$ 0.19
Parietal cortex	1.76 $\pm$ 0.17	1.65 $\pm$ 0.15	1.51 $\pm$ 0.16
Auditory cortex	2.49 $\pm$ 0.14	2.42 $\pm$ 0.22	2.06 $\pm$ 0.27
Visual cortex	1.27 $\pm$ 0.13	1.19 $\pm$ 0.10	1.06 $\pm$ 0.08
Caudate-putamen	1.44 $\pm$ 0.09	1.33 $\pm$ 0.13	1.18 $\pm$ 0.11
Hypothalamus	0.93 $\pm$ 0.06	0.97 $\pm$ 0.09	0.97 $\pm$ 0.09
Hippocampus	0.76 $\pm$ 0.04	0.78 $\pm$ 0.05	0.77 $\pm$ 0.05
Amygdala	0.88 $\pm$ 0.05	0.94 $\pm$ 0.07	0.95 $\pm$ 0.07
Habenula	1.57 $\pm$ 0.11	1.37 $\pm$ 0.08	1.26 $\pm$ 0.07
Substantia nigra	0.99 $\pm$ 0.09	1.01 $\pm$ 0.10	0.93 $\pm$ 0.08
Medial geniculate	1.79 $\pm$ 0.11	1.68 $\pm$ 0.18	1.54 $\pm$ 0.16
Lateral geniculate	1.45 $\pm$ 0.15	1.26 $\pm$ 0.11	1.23 $\pm$ 0.09
Superior colliculus	1.56 $\pm$ 0.09	1.34 $\pm$ 0.10	1.20 $\pm$ 0.07
Inferior colliculus	1.79 $\pm$ 0.13	1.98 $\pm$ 0.23	1.85 $\pm$ 0.15
Red nucleus	1.46 $\pm$ 0.10	1.24 $\pm$ 0.13	1.12 $\pm$ 0.17
Superior olive	1.55 $\pm$ 0.09	1.50 $\pm$ 0.19	1.38 $\pm$ 0.18

The values are means $\pm$ S.E.M. No significant differences were observed in any of the structures between lesion side and control side, lesion side and non-lesioned, and between control side and non-lesioned.

However, the CBF values in the various structures on the lesion side were not statistically different from those measured on the control side or in the non-lesioned animal. Since these results were in agreement with those for normocapnic lesioned animals presented by Lindvall et al.²⁹, the materials were pooled (Table 2).

Table 1 gives the physiological parameters and Table 3 the local CBF values in hypercapnic, 6-OHDA-lesioned animals. Hypercapnia increased CBF 2-3 fold in all structures (compare Tables 2 and 3). This increase is in the same order of magnitude as that measured with the local CBF technique in non-lesioned hypercapnic animals (compare the present values in normocapnia (Table 2 with those measured in hypercapnia by Dahlgren et al., in preparation). No statistically significant differences in CBF between lesion side and control side were observed in any of the structures. However, in the caudate-putamen mean CBF during hypercapnia was about 10 per cent higher on the lesion side. This should be compared with the

Table 3. Local CBF ($\text{ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$) at hypercapnia in rats after unilateral 6-hydroxydopamine lesion of the nigrostriatal dopamine bundle.

Structures	6-OHDA-lesioned (n=6)	
	Control side	Lesion side
Frontal cortex	3.65 $\pm$ 0.13	3.75 $\pm$ 0.18
Sensorimotor cortex	4.16 $\pm$ 0.21	4.19 $\pm$ 0.24
Parietal cortex	3.86 $\pm$ 0.22	3.69 $\pm$ 0.22
Auditory cortex	4.86 $\pm$ 0.23	4.71 $\pm$ 0.24
Visual cortex	2.54 $\pm$ 0.04	2.54 $\pm$ 0.09
Caudate-putamen	3.32 $\pm$ 0.16	3.63 $\pm$ 0.20
Hypothalamus	2.22 $\pm$ 0.07	2.38 $\pm$ 0.10
Hippocampus	2.42 $\pm$ 0.12	2.49 $\pm$ 0.11
Amygdala	2.42 $\pm$ 0.12	2.62 $\pm$ 0.11
Habenula	3.83 $\pm$ 0.15	4.00 $\pm$ 0.17
Substantia nigra	2.45 $\pm$ 0.12	2.45 $\pm$ 0.11
Medial geniculate	4.29 $\pm$ 0.18	4.29 $\pm$ 0.16
Lateral geniculate	3.39 $\pm$ 0.12	3.64 $\pm$ 0.17
Superior colliculus	3.84 $\pm$ 0.11	3.85 $\pm$ 0.11
Inferior colliculus	4.71 $\pm$ 0.14	4.83 $\pm$ 0.10
Red nucleus	3.05 $\pm$ 0.17	2.99 $\pm$ 0.13
Superior olive	3.59 $\pm$ 0.20	3.53 $\pm$ 0.22

The values are means $\pm$ S.E.M. No significant differences were observed in any of the structures between lesion side and control side.

CBF values in normocapnia which are about 10 per cent lower on the lesion side. Table 4 shows the ratio between CBF on the

Table 4. Effects of hypercapnia on local CBF in rats with unilateral 6-hydroxydopamine lesions of the nigrostriatal dopamine bundle.

Structures	Local CBF ratio	
	Normocapnia (n=9)	Hypercapnia (n=6)
Frontal cortex	0.92 $\pm$ 0.05	1.01 $\pm$ 0.03
Sensorimotor cortex	0.86 $\pm$ 0.06	1.01 $\pm$ 0.03
Caudate-putamen	0.91 $\pm$ 0.04	1.11 $\pm$ 0.02 ^{xxx}
Hypothalamus	1.00 $\pm$ 0.02	1.07 $\pm$ 0.02
Amygdala	1.02 $\pm$ 0.02	1.08 $\pm$ 0.02
Substantia nigra	0.94 $\pm$ 0.05	1.00 $\pm$ 0.03

The values are means $\pm$ S.E.M. of ratio $\frac{\text{measured value lesion side}}{\text{measured value control side}}$.

xxx $p < 0.001$.

lesion side and that on the control side in various structures at normocapnia and hypercapnia. There was a highly significant increase of this ratio in the caudate-putamen at hypercapnia whereas no significant change was observed in any other structure. The results thus indicate that in animals with unilateral lesions of the dopaminergic nigrostriatal bundle the increase of CBF induced by hypercapnia is more pronounced in the denervated caudate-putamen. In contrast, the CBF response to hypercapnia seemed unchanged in other brain regions (frontal cortex and amygdala) which were also deprived of their mesencephalic dopaminergic input.

B. Effects of lesions of 5-hydroxytryptamine-containing pathways.

Table 5 shows the 5-HT content in the cortex and brain stem 2 weeks after the 5,7-DHT injections or sham lesions had been performed.

Table 5. Levels of 5-hydroxytryptamine in the cortex and brain stem 14 days after Intraventricular 5,7-dihydroxytryptamine.

Experimental groups	Frontal parietal cortex	Pons + medulla oblongata
Sham operated	375.1 $\pm$ 42.3 (7)	459.0 $\pm$ 15.4 (7)
Lesioned, normo-capnia	32.3 $\pm$ 12.5 ^{XXX} (6)	220.1 $\pm$ 5.1 ^{XXX} (4)
Lesioned, hypercapnia	28.5 $\pm$ 8.6 ^{XXX} (8)	261.2 $\pm$ 14.8 ^{XXX} (8)

The values are means $\pm$ S.E.M. The asterisks indicate significant differences ($p < 0.001$) compared to the sham operated group. No significant differences were observed between the lesioned groups. Number of experimental animals taken for analysis is given in brackets.

In agreement with Carlsson et al.⁹ there was no significant difference in 5-HT content between normocapnic and hypercapnic brains and therefore the samples from the two sham-operated

groups were pooled. In the 5,7-DHT lesioned animals there was a decrease in 5-HT content in the cortex by 91 per cent (in the normocapnic group) and 92 per cent (in the hypercapnic group), and in the brain stem by 52 per cent and 43 per cent, respectively. Treatment with 5,7-DHT has no lesion effects on dopaminergic neurons² and since, in the present experiments, desipramine was administered prior to 5,7-DHT even the noradrenergic neurons should have been protected against its neurotoxic actions⁶.

Table 6 gives the physiological parameters and CBF/CMRO₂ in normocapnic controls and in sham operated and 5,7-DHT lesioned animals 2 weeks after surgery.

Table 6. Physiological parameters and CBF/CMR_{O₂} in normocapnic animals: Influence of 5,7-dihydroxytryptamine-induced lesions of central serotonergic neuron systems.

Experimental groups	Temp °C	MABP mm Hg	PaO ₂ mm Hg	PaCO ₂ mm Hg	Arterial pH	CaO ₂ μmol·ml ⁻¹	AVD _{O₂} ml·g ⁻¹ ·min ⁻¹	CBF ml·g ⁻¹ ·min ⁻¹	CMR _{O₂} μmol·g ⁻¹ ·min ⁻¹
Control (n=7)	37.1 ±0.1	149 ±4	108 ±3	34.8 ±0.8	7.39 ±0.01	10.63 ±0.17	3.66 ±0.22	1.10 ±0.11	3.94 ±0.28
Sham operated (n=4)	37.1 ±0.1	145 ±2	108 ±1	38.1 ±1.6	7.39 ±0.02	10.82 ±0.18	2.77 ±0.54	1.79 ±0.39	4.37 ±0.26
Lesioned (n=6)	37.2 ±0.2	148 ±2	106 ±1	36.2 ±0.9	7.38 ±0.02	10.33 ±0.16	3.01 ±0.41	1.75 ±0.19	4.84 ^x ±0.24

The values are means ±S.E.M. The asterisk indicates significant difference ($p < 0.05$) compared to the control group. No other significant differences in any of the parameters studied were observed between the various groups.

As compared with non-lesioned controls mean CBF was higher both in the sham operated and the 5,7-DHT lesioned animals. However, no statistically significant difference was observed between the CBF values of the various experimental groups. Furthermore, since the control animals were not studied during the same time period as the other groups, it cannot be concluded that the sham operation or 5,7-DHT lesion leads to an increase in CBF (see also the PaCO₂ values). However, the 5,7-DHT induced change in CMR_{O₂} seems so large that it probably reflects a true increase in metabolic rate caused by removal of the 5-HT innervation (cf. Harper and MacKenzie²¹). Mean

CMR_{O₂} was about 10 per cent higher in the 5,7-DHT treated animals than in the sham operated group and in comparison with the control group the increase in CMR_{O₂} (about 23 per cent) reached statistical significance.

In order to reveal any time dependent changes in CBF and CMR_{O₂} after the 5,7-DHT injections, some animals were analyzed 3 days after operation. No significant differences between rats with 3 and 14 day-old lesions could be observed. It therefore seems less likely that receptor supersensitivity had any influence on the results (cf. Edvinsson and MacKenzie¹⁶, and Wang et al.⁴⁵).

Table 7 shows the physiological parameters and CBF/CMR_{O₂} at hypercapnia in controls and in animals 2 weeks after sham operation or 5,7-DHT injection.

Table 7. Physiological parameters and CBF/CMR_{O₂} in hypercapnic animals: Influence of 5,7-dihydroxytryptamine-induced lesions of central serotonergic neuron systems.

Experimental groups	Temp	MABP mm Hg	Pa _{O₂} mm Hg	Pa _{CO₂} mm Hg	Arterial pH	Ca _{O₂} μmol·ml ⁻¹	AVD _{O₂} ml·g ⁻¹ ·min ⁻¹	CBF ml·g ⁻¹ ·min ⁻¹	CMR _{O₂} μmol·g ⁻¹ ·min ⁻¹
Control (n=8)	36.7 ±0.1	154 ±5	121 ±3	84 ±2	7.13 ±0.01	9.91 ±0.33	0.85 ±0.07	5.43 ±0.51	4.52 ±0.36
Sham operated (n=4)	37.0 ±0.1	140	116 ±6	77 ^x ±2	7.19 ^{xxx} ±0.01	9.66 ±0.17	1.07 ±0.12	4.49 ±0.54	4.62 ±0.05
Lesioned (n=8)	36.8 ±0.1	146 ±1	109 ^{xx} ±1	77 ^{xx} ±1	7.16 ±0.02	9.38 ±0.23	0.91 ±0.09	5.43 ±0.54	4.74 ±0.43

The values are means ± S.E.M. The asterisks indicate significant differences (^x denotes $p < 0.05$, ^{xx} $p < 0.01$, and ^{xxx} $p < 0.001$) compared to control group. In addition MABP was higher ($p < 0.05$) in lesioned compared to sham operated animals. No other significant differences in any of the parameters studied were observed between the various groups.

No significant differences in CBF or CMR_{O₂} were observed between the various experimental groups, indicating that the 5-HT systems are not involved in the cerebral circulatory response to hypercapnia. It is noteworthy that hypercapnia induced a slight though not significant, increase in CMR_{O₂} (cf. Berntman et al.⁵) both in the control and shamoperated groups but that no change in CMR_{O₂} was observed in 5,7-DHT lesioned animals.

DISCUSSION

Despite a large number of experimental studies (for references see e.g. Edvinsson and MacKenzie¹⁶) the involvement of nervous mechanisms in the cerebrovascular CO₂ response is still much debated. No major role for the well-established sympathetic and parasympathetic innervations of the brain vessels has hitherto been disclosed. Since lesions in the pons can attenuate the vasodilatation accompanying an increased PCO₂, it has been suggested that this response is regulated via a brain stem center⁴². Some drugs that interfere with noradrenergic neurotransmission have the same effect^{5,31}, and it therefore seemed possible that the locus coeruleus, which is located in the pons, is identical with this brain stem center, exerting its circulatory effects via vasomotor fibres thus innervating the cerebral microvessels²³. This hypothesis has been corroborated by Bates et al.¹ who found that electrolytic lesions of the locus coeruleus in the cat markedly reduced the change in CBF in response to hypercapnia. However, we have recently shown¹¹ that in the rat 6-hydroxydopamine lesions of the ascending projections from the locus coeruleus have no effect on the CO₂ sensitivity of the cerebral circulation. Since our lesions spared projections to the lower brain stem and spinal cord a possible influence of the locus coeruleus system on the CO₂ response to hypercapnia could still be mediated via these connections.

The present study has demonstrated that even selective lesions of a central dopaminergic neuron system lack major effects on CBF in normocapnia and hypercapnia. This is in good agreement with the study of McCulloch and Harper³² where administration of the dopamine receptor blocking agent pimozide was found to cause no change in CBF under these conditions. However, the results of the lesions indicate that the nigrostriatal dopaminergic system has a minor modulatory influence on the circulatory response to hypercapnia in the caudate-putamen. At normocapnia the 6-hydroxydopamine lesion of the ascending dopamine bundle leads to a slight (about 10 per cent) decrease in blood flow in the denervated striatum.

This change is in the same order of magnitude as the decrease in 2-deoxyglucose uptake found after such a lesion^{25,38,40}. It thus seems probable that the change in blood flow observed under normocapnic conditions is secondary to a decrease in metabolic rate. At hypercapnia the blood flow is slightly (about 10 per cent) higher in the denervated caudate-putamen compared with the control side. If the ratio between CBF on the two sides is compared at normocapnia and hypercapnia (Table 4) it is found to increase significantly in the caudate-putamen under hypercapnic conditions. This indicates that the circulatory response to hypercapnia is enhanced in the denervated caudate-putamen. No such effects can be observed in any other brain region including those which were deprived of their dopaminergic input by the lesion.

The background to this change in CO₂ reactivity of the circulation in the denervated striatum is unclear. Local metabolic changes induced by hypercapnia in the lesioned animals have not yet been investigated. However, it is tempting to speculate that under hypercapnic conditions the dramatic increase in blood flow in the caudate-putamen is modulated by a slight vasoconstriction mediated via the nigrostriatal dopamine system. The amphetamine-induced changes in striatal circulation after removal of the dopaminergic input indicate that release of endogenous dopamine leads to a reduction in blood flow²⁹. This has also been concluded from experiments with electrical stimulation of the substantia nigra²⁷. If this is the case, dopamine release from intact nigrostriatal axons under hypercapnic conditions should reduce blood flow in the caudate-putamen. It should be recalled, however, that the study of DeYebenes Prous et al.¹³ have indicated that the impulse flow in dopaminergic neurons is decreased in hypercapnia.

The proposed serotonergic innervation of forebrain microvessels originates mainly in the nucleus raphe dorsalis and medianus³⁷. The cortical projections of these brain stem nuclei were efficiently removed by the 5,7-DHT injections as indicated by about 90 per cent decrease in 5-HT levels. The Kety-Schmidt technique, which measures blood flow primarily in frontal and parietal cortical areas, could reveal no effect

of this 5-HT depletion on the circulatory response to hypercapnia. No difference in CBF was observed between animals with more than 95 per cent reduction in cortical 5-HT and those with 85-90 per cent depletion. Although the results thus indicate that the serotonergic vascular innervation is not involved in the circulatory response to hypercapnia in frontal and parietal cortex it cannot be excluded that it affects the blood flow under these conditions in other parts of the brain.

Numerous studies have shown that intracerebral serotonergic systems inhibit neuronal activity in various regions of the brain (see for example Segal⁴¹). Furthermore, administration of 5-HT after osmotic disruption of the blood brain barrier leads to a reduction of both cerebral oxygen and glucose consumption²¹. It is therefore interesting that the 5,7-DHT lesions seemed to induce a slight increase in cerebral metabolic rate under normocapnic conditions. This indicates that the serotonergic system exerts a depressant resting tone on metabolic rate in the brain.

Neither our previous study¹¹ nor the present one have disclosed any major involvement of central noradrenergic, dopaminergic and serotonergic neuron systems in the cerebral circulatory response to hypercapnia. It must be point out that our data do not exclude that these systems innervate microvessels in the brain and that they participate in the control of cerebrovascular tone in other conditions than those studied presently. However, the functional states when these intrinsic vasoregulatory systems are of any real importance still remain to be defined.

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THE EFFECT OF PROPRANOLOL ON LOCAL CEREBRAL BLOOD FLOW
UNDER NORMOCAPNIC AND HYPERCAPNIC CONDITIONS

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ABSTRACT

The effect of propranolol ($2.5 \text{ mg} \cdot \text{kg}^{-1}$ i.v.) on local CBF in normocapnia was studied in rats maintained artificially ventilated on 70% N_2O and 30% O_2 . Although a single dose of propranolol, given 30 min prior to CBF measurements, reduced mean CBF values in all of the 22 structures analysed, none of the changes were significant. The results confirm previous ones, in which overall CBF was measured, in showing that β -adrenergic mechanisms have little effect on normal cerebrovascular tone.

Following a single dose of propranolol, results obtained in hypercapnia were equally negative. Significant reductions in CBF (in 5 of 22 structures) were obtained only when propranolol was given by constant infusion during 15 min. However, the effects showed no obvious relationship to the distribution of β -adrenoceptors in the brain. It is concluded that the reduction in CBF during hypercapnia, observed in previous studies, is secondary to metabolic depression, and that these effects reflect nonspecific, local anaesthetic effects rather than a specific inhibition of β -adrenergic receptors.

It has been known for more than 50 years that cerebral arteries are innervated and that part of this innervation originates from the cervical sympathetic chain (e.g. Stöhr 1922, Forbes 1928). Attempts to unravel the physiological role of this innervation by section or stimulation of the sympathetic nerves gave essentially negative results since changes in cerebral blood flow (CBF) were either small or undetectable (for literature, see Schmidt 1950, Sokoloff 1959). With the advent of modern histofluorescence techniques, and the demonstration that the innervation extends to intraparenchymal vessels (see Edvinsson and MacKenzie 1976, Owman et al. 1977), the interest in neurogenic control of CBF was rejuvenated. As a result, it has been demonstrated that the innervation involves α -adrenoceptors and that the effects of sympathetic stimulation can be mimicked by α -receptor antagonists (Lowe and Gilboe 1971, Kuschinsky et al. 1973, Oberdörster et al. 1973, Rosendorff et al.

1976). There is little indication, though, that the extrinsic sympathetic innervation is responsible for normal cerebrovascular tone, or that it constitutes an important mechanism of CBF regulation. An attractive explanation of the role of the innervation was suggested by Harper et al. (1972) in their 'series resistance' hypothesis. According to this, neurogenic constriction or dilatation of proximal, innervated arteries will contribute to overall cerebrovascular resistance. However, in all but some extreme circumstances this does not affect CBF since other, non-neurogenic mechanisms regulate the tone of more distal resistance vessels.

In recent years, interest has been redirected towards the possible role played by an intrinsic innervation of cerebral microvessels by noradrenergic fibres originating in the locus coeruleus and other brainstem nuclei. This interest was stimulated by demonstrations of such nerves in close association with cerebral microvessels (Hartman et al. 1972, Rennels and Nelson 1975, Swanson et al. 1977), and by results suggesting that stimulation or inhibition of central noradrenergic activity affects CBF and vascular permeability (Raichle et al. 1975, Grubb et al. 1978). Recent results suggest that any such effects should be mediated via β -adrenoceptors, localized to intracranial "microvessels" (Herbst et al. 1979, Nathanson and Glaser 1979, Harik et al. 1980).

Results of the type discussed raise the question of the physiological role played by a β -adrenoceptor innervation. By administration of appropriate blockers, usually propranolol, several groups have attempted to study whether such a system contributes to normal cerebrovascular tone, or modulates the CBF response to hypercapnia. This type of analysis is complicated by the fact that β -receptors occur in neurons and glial cells as well, and that β -blocking agents may influence both neuronal activity (for literature, see Bloom 1975) and cellular metabolism (see Nahorski et al. 1975). Most authors have attempted, therefore, to measure both CBF and metabolic rate. The results are inconclusive and partly contradictory. Thus, some authors have found that propranolol does not affect CBF under control conditions (MacKenzie et al. 1976, Berntman et al. 1978, Olesen et al. 1978), others that it reduces CBF (Aoyagi

et al. 1976). In some studies, propranolol was found to reduce metabolic rate (Aoyagi et al. 1976, MacKenzie et al. 1976, Savaki et al. 1978) but in another, CMR_{O_2} was not changed from control (Berntman et al. 1978). Four groups of workers have reported that propranolol reduces CBF under hypercapnic condition (Aoyagi et al. 1976, MacKenzie et al. 1976, Berntman et al. 1979, Hemmingsen et al. 1979), but a fifth group found no such effect (Olesen et al. 1978).

The objective of the present experiments was to re-evaluate the effect of propranolol on CBF under normocapnic and hypercapnic conditions. As will be discussed below, we had reasons to suspect that propranolol rapidly dissociates from its receptor binding sites, and that its effects on CBF and metabolic rate are transient. We, therefore, measured CBF both during constant infusion and 30 min following a single dose of propranolol. Furthermore, since it has been suggested that the effect of β -adrenergic stimulation are regionally different (Aubineau et al. 1973, see also Sercombe et al. 1977, Savaki et al. 1978) CBF was studied by autoradiographic techniques. The dose of propranolol employed ($2.5 \text{ mg} \cdot \text{kg}^{-1}$) is one that is not assumed to induce nonspecific, local anaesthetic effects but nevertheless sufficient to block effects of β -adrenoreceptor agonists in the brain (see Nahorski et al. 1975, Berntman et al. 1978).

MATERIALS AND METHODS

Animals: All experiments were performed on male Wistar rats (SPF strain, Møllegaards Avlslaboratorium, Copenhagen, Denmark) weighing between 305 and 415 g. The animals had free access to tap water and food pellets (Astra-Ewos, Södertälje, Sweden) until operation.

Drugs: Propranolol $1 \text{ mg} \cdot \text{ml}^{-1}$ (standard racemic solution, Inderal^(R), ICI, England), and d-tubocurarine $3 \text{ mg} \cdot \text{ml}^{-1}$ (Tubocurarin^(R), Vitrum, Stockholm, Sweden) were used in stock solutions as provided. Heparin (Vitrum, Stockholm, Sweden) was diluted by Krebs-Hensleit solution to contain $300 \text{ I.U.} \cdot \text{ml}^{-1}$. ^{14}C -hexadecane (standard solution) and ^{14}C -iodoantipyrine were

obtained from the Radiochemical Centre (Amersham, England). The ^{14}C -iodoantipyrine was diluted by ethanol to contain $100\ \mu\text{Ci}\cdot\text{ml}^{-1}$. Aliquots of 0.5 and 0.3 ml of this solution were used for normocapnic and hypercapnic experiments, respectively. The ethanol was evaporated in a stream of nitrogen and the substance dissolved in 1.5 (normocapnic) and 0.7 (hypercapnic) ml of Krebs-Hensleit solution.

Operative and sampling techniques: The animals were anaesthetized with 4% halothane in $\text{N}_2\text{O}/\text{O}_2$ (70:30). When unresponsive the animals were tracheotomized and connected to a Starling type respirator delivering 1% halothane in $\text{N}_2\text{O}/\text{O}_2$ (70:30). Paralysis was accomplished by i.v. injection of d-tubocurarine, $1.5\ \text{mg}\cdot\text{kg}^{-1}$. One brachial artery was cannulated with a catheter 15 mm in length, used for blood sampling for tracer concentration determination. A femoral artery and vein were cannulated to obtain continuous blood pressure recording, anaerobic sampling for blood gas and pH determination (Eschweiler and Co., Kiel, W.Germany, and Radiometer, Copenhagen, Denmark), and for injection of drugs and tracer substance. Temperature was controlled at about 37°C by means of external heating. After completion of the operation the halothane supply was discontinued, the animals were then heparinized ($300\ \text{I.U.}\cdot\text{kg}^{-1}$) and left to equilibrate for 30 min.

Induction of hypercapnia: By substituting 5-6% of the N_2O for CO_2 hypercapnia (PaCO_2 about 75 mm Hg) was induced.

Administration of propranolol: Propranolol $2.5\ \text{mg}\cdot\text{kg}^{-1}$ was administered i.v. in two different ways, as a single dose or as continuous infusion. When given as a single dose, the drug was injected over about 2 min in order to avoid circulatory disturbances. This way of administration was used for two experimental groups, one normocapnic and one hypercapnic. In these, hypercapnia was induced 5 min after the injection. Local-CBF was determined 30 min after the injection of propranolol. In one hypercapnic group, propranolol ($2.5\ \text{mg}\cdot\text{kg}^{-1}$) was infused at constant rate for 15 min before 1-CBF was measured. In these animals, hypercapnia was induced 3 min after the start of the infusion.

Determination of local CBF: Local-CBF was measured according to Sakurada et al. (1978) with ^{14}C -iodoantipyrine as diffusible tracer (for details, see Abdul-Rahman et al. 1979). However, the technique was modified in two respects. Thus infusion of tracer substance was limited to 20 sec during hypercapnia, and blood for determination of ^{14}C -activity was collected from the brachial artery. ^{14}C -activity was determined by liquid scintillation (Rackbeta 1215, LKB Wallac, Turku, Finland) the efficiency in counting being controlled with an internal standard (^{14}C -hexadecane). Optical density was measured with a densitometer with an aperture of 1 mm^2 (MacBeth TD501, Newburgh, N.Y., USA).

RESULTS

As stated, three series of animals were studied, all given propranolol in a dose of $2.5\text{ mg}\cdot\text{kg}^{-1}$: one normocapnic group and two hypercapnic, the latter differing only in the mode of administration of propranolol (single dose given 30 min before CBF determination, and constant infusion during 15 min, respectively). The values were compared to those obtained in control animals not given propranolol (normocapnic and hypercapnic, see Dahlgren et al. 1981). We obtained the latter values in experiments run in parallel to those in which propranolol was employed, using identical techniques (in all experiments, the CBF was measured by N.D. and M.I.).

Table 1 shows the physiological parameters. The values were sufficiently similar in the normocapnic and hypercapnic groups, respectively, to exclude the possibility that differences in body temperature, mean arterial blood pressure, or PaCO_2 could have influenced the results.

1. CBF technique

In preliminary experiments, l-CBF was measured during hypercapnia according to Abdul-Rahman et al. (1979), i.e. with 30 sec infusion of isotope and sampling of arterial blood from the femoral artery. This procedure was found to yield l-CBF values that were excessively high and inconsistent. In order to improve accuracy at those high flow rates two modifications were made. First, in order to obtain blood with minimal

Table 1. Physiological parameters for controls and animals given propranolol under normocapnia and hypercapnia.

Experimental group	Temp °C	MABP mm Hg	PaCO ₂ mm Hg	PaO ₂ mm Hg	pH
I Normocapnic controls n=6	37.3 +0.4 ±0.2	144 +10 ±4	38.1 ±2.4 ±1.0	103 +5 ±2	7.42 +0.04 ±0.02
II Normocapnic propranolol n=6	37.1 +0.5 ±0.2	161 ±9 ±4	37.4 +3.1 ±1.3	105 ±6 ±3	7.39 +0.03 ±0.01
III Hypercapnic controls n=6	37.1 +0.3 ±0.1	141 ±7 ±3	76 +3 ±1	109 +5 ±2	7.18 +0.02 ±0.01
IV Hypercapnic propranolol n=6	36.8 +0.3 ±0.1	139 ±8 ±3	76 +4 ±2	114 +4 ±2	7.15 +0.04 ±0.02
V Hypercapnic propranolol n=5	36.6 +0.3 ±0.1	140 ±0 ±1	75 +3 ±1	112 +6 ±3	7.18 +0.03 ±0.01

MABP = mean arterial blood pressure. For groups II and IV propranolol (2.5 mg·kg⁻¹) was given as a single injection 30 min before I-CBF determination while for group V the same dose was given continuously during 15 min preceding I-CBF measurement. (Group I from Dahlgren et al. 1981) Values are means ± S.E.M.

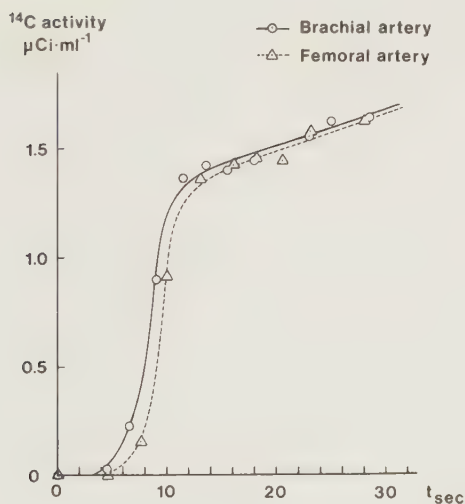


Fig. 1. The rise in ¹⁴C-activity in blood collected from the brachial (circles) and femoral (triangles) artery during the infusion of 50 µCi of ¹⁴C-iodoantipyrine in hypercapnia.

temporal distortion of the ^{14}C -activity curve a short brachial, rather than a femoral artery catheter was employed. As fig. 1 shows the rate of rise of ^{14}C -activity differed when blood was sampled from the brachial and femoral artery, the rise occurring about 0.75 sec earlier with sampling from the brachial artery. Second, in order to obtain better resolution of the brain activity versus blood activity at the end of the infusion (see Eklöf et al. 1974) the sampling time was shortened to 20 sec, with sampling every 2 sec. With these modifications, reproducible results were obtained even at flow rates of $5\text{--}7\text{ ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$. Since the procedure does not significantly influence CBF values under normocapnic conditions, we conclude that, by minimizing the delay caused by withdrawal of blood from a peripheral artery, the hypercapnic CBF values are better estimates of the true CBF values. However, since the procedure does not eliminate any errors due to diffusion limitation of the isotope (see Eklöf et al. 1974, Eckman et al. 1975), the true values in hypercapnia are probably somewhat higher than reported here. Since any such error should be equally pronounced in all three experimental groups subjected to hypercapnia it cannot affect the conclusions drawn.

2. Effect of propranolol on CBF under normocapnic conditions

Table 2 gives local CBF in 22 different structures in control (Dahlgren et al. 1981) and propranolol-injected animals ($2.5\text{ mg}\cdot\text{kg}^{-1}$ given 30 min prior to CBF measurement). Propranolol did not change CBF significantly in any of the structures analysed. However, the mean values were lower than control in 21 of 22 structures, and if one grossly aberrant cerebellar value was excluded ($2.54\text{ ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$), propranolol reduced CBF in all. Thus by pooling of data one finds that propranolol appeared to reduce CBF by 10–15%. Such a small reduction is consistent with previous data on overall (cortical) CBF (Berntman et al. 1978). As the results demonstrate, though, the suggested reduction in CBF was uniform with no preferential localization to certain structures.

3. Effect of propranolol on CBF in hypercapnia.

The corresponding results for hypercapnic animals are given in table 3. In view of previous results (MacKenzie et al. 1976, Berntman et al. 1979) the results obtained with a single dose

Table 2. Local cerebral blood flow in controls and animals given propranolol.

Structure	Controls n=6	Propranolol n=6
Frontal cortex	1.58 $\pm$ 0.20	1.30 $\pm$ 0.10
Nc. ruber	1.46 $\pm$ 0.10	1.19 $\pm$ 0.08
Striatum	1.44 $\pm$ 0.09	1.33 $\pm$ 0.11
Substantia nigra	0.99 $\pm$ 0.09	0.91 $\pm$ 0.06
Cerebellum	0.92 $\pm$ 0.10	1.12 $\pm$ 0.29
Sensory-motor cortex	1.69 $\pm$ 0.20	1.46 $\pm$ 0.13
Parietal cortex	1.76 $\pm$ 0.17	1.46 $\pm$ 0.14
Thalamus	1.33 $\pm$ 0.08	1.16 $\pm$ 0.06
Nc. anterior thalami	1.41 $\pm$ 0.09	1.29 $\pm$ 0.09
Nc. vent.post. thalami	1.56 $\pm$ 0.11	1.28 $\pm$ 0.05 ^x
Visual cortex	1.27 $\pm$ 0.13	1.16 $\pm$ 0.10
Lat. geniculate body	1.45 $\pm$ 0.15	1.13 $\pm$ 0.05
Superior colliculus	1.56 $\pm$ 0.09	1.33 $\pm$ 0.10
Auditory cortex	2.49 $\pm$ 0.14	2.24 $\pm$ 0.19
Med. geniculate body	1.79 $\pm$ 0.11	1.47 $\pm$ 0.09
Inferior colliculus	1.79 $\pm$ 0.13	1.60 $\pm$ 0.13
Superior olive	1.55 $\pm$ 0.09	1.36 $\pm$ 0.09
Hippocampus	0.76 $\pm$ 0.04	0.73 $\pm$ 0.03
Amygdala	0.88 $\pm$ 0.05	0.77 $\pm$ 0.05
Septal nuclei	0.77 $\pm$ 0.04	0.70 $\pm$ 0.04
Habenula	1.57 $\pm$ 0.11	1.38 $\pm$ 0.09
Hypothalamus	0.93 $\pm$ 0.06	0.85 $\pm$ 0.07

Propranolol (2.5 mg·kg⁻¹) was given as a single dose 30 min prior to I-CBF measurement.

Values are means $\pm$ S.E.M. ^x denotes p < 0.05.

(Control material from Dahlgren et al. 1981)

of propranolol were surprising. Thus, in none of the structures analysed did propranolol significantly reduce hypercapnic flow rates. Suspecting that this was due to dissociation of propranolol from binding sites, we infused the drug during 15 min. As the results show, this procedure caused CBF to fall in some structures (frontal and sensorimotor cortex, ventral-posterior parts of the thalamus, substantia nigra, and superior olive). Furthermore, in most of the remaining structures the mean values were reduced, however not to a statistically significant level, when comparing with uninjected hypercapnic animals.

Table 3. Local cerebral blood flow in controls and animals given propranolol.

Structure	Control	Propranolol single dose	Propranolol continuously
Frontal cortex	4.43 $\pm$ 0.13	4.28 $\pm$ 0.24	3.94 $\pm$ 0.11 ^x
Nc ruber	3.93 $\pm$ 0.40	3.51 $\pm$ 0.15	3.21 $\pm$ 0.16
Striatum	4.18 $\pm$ 0.25	3.66 $\pm$ 0.19	3.60 $\pm$ 0.25
Substantia nigra	3.18 $\pm$ 0.24	2.58 $\pm$ 0.11 ^x	2.51 $\pm$ 0.11 ^x
Cerebellum	2.76 $\pm$ 0.22	2.92 $\pm$ 0.09	2.73 $\pm$ 0.14
Sensory-motor cortex	5.65 $\pm$ 0.17	4.66 $\pm$ 0.39 ^x	4.57 $\pm$ 0.37 ^x
Parietal cortex	4.75 $\pm$ 0.21	4.54 $\pm$ 0.44	4.35 $\pm$ 0.41
Thalamus	4.26 $\pm$ 0.20	3.92 $\pm$ 0.17	3.73 $\pm$ 0.20
Nc anterior thalami	5.30 $\pm$ 0.52	5.10 $\pm$ 0.29	4.44 $\pm$ 0.18
Nc vent.post.thalami	5.08 $\pm$ 0.22	4.44 $\pm$ 0.30	3.85 $\pm$ 0.25 ^{xx}
Visual cortex	2.68 $\pm$ 0.09	2.97 $\pm$ 0.32	3.19 $\pm$ 0.28
Lat.geniculate body	4.26 $\pm$ 0.29	3.96 $\pm$ 0.24	3.76 $\pm$ 0.21
Superior colliculus	5.07 $\pm$ 0.65	4.33 $\pm$ 0.14	3.83 $\pm$ 0.19
Auditory cortex	6.03 $\pm$ 0.30	5.73 $\pm$ 0.24	5.00 $\pm$ 0.42
Med.geniculate body	6.08 $\pm$ 0.55	5.16 $\pm$ 0.24	4.91 $\pm$ 0.26
Inferior colliculus	5.80 $\pm$ 0.54	5.78 $\pm$ 0.49	5.37 $\pm$ 0.37
Superior olive	4.79 $\pm$ 0.47	4.08 $\pm$ 0.19	3.28 $\pm$ 0.21 ^{x†}
Hippocampus	2.46 $\pm$ 0.19	2.54 $\pm$ 0.12	2.60 $\pm$ 0.18
Amygdala	2.86 $\pm$ 0.24	2.51 $\pm$ 0.14	2.49 $\pm$ 0.16
Septal nuclei	2.13 $\pm$ 0.13	2.12 $\pm$ 0.11	2.26 $\pm$ 0.18
Habenula	4.94 $\pm$ 0.43	4.50 $\pm$ 0.21	4.12 $\pm$ 0.22
Hypothalamus	2.51 $\pm$ 0.25	2.34 $\pm$ 0.12	2.24 $\pm$ 0.14

Propranolol (2.5 mg·kg⁻¹) was given as a single dose 30 min before I-CBF determination in animals designated "propranolol single dose". The same dose was given as a continuous infusion during 15 min preceding I-CBF measurement in animals designated "propranolol continuously". Values are means $\pm$ S.E.M. ^x denote p < 0.05, ^{xx} p < 0.01 when comparing to controls and [†] denotes p < 0.05 when comparing between groups given propranolol.

DISCUSSION

The present results demonstrate that propranolol in a dose of 2.5 mg·kg⁻¹ has but small effects on local CBF. The results obtained in normocapnic animals (a suggested but nonsignificant decrease by about 10%) confirm those of MacKenzie et al. (1976), Olesen et al. (1978) and Berntman et al. (1978). The combined results make it very unlikely that a β -adrenergic influence helps to maintain cerebrovascular tone. In this respect, the results are in close agreement with our previous ones, demonstrating that lesions of the noradrenergic system originating from the nucleus locus coeruleus fail to modify CBF (Dahlgren et al. 1981b).

In view of the results obtained in previous studies (Aoyagi et al. 1976, MacKenzie et al. 1976, Berntman et al. 1979, Hemmingsen et al. 1979) it is perhaps more surprising that a single dose of propranolol did not modify the CBF response to hypercapnia, but the results corroborate those of Olesen et al. (1978). Conflicting results have been published regarding the duration of action of propranolol. Thus, whereas Bylund and Snyder (1976) have noted a relatively fast dissociation of Alprenolol, a β -receptor antagonist analogue, from its receptor binding sites, and Olesen et al. (1978) found rapid influx and egress of propranolol in studies designed to investigate the extraction of the drug from arterial blood, other results have been published that suggest that propranolol remains bound to cerebral receptors for several hours (Hayes and Cooper 1971, Evans et al. 1973 and Elghozi et al. 1979).

Suspecting that propranolol dissociates from (vascular?) receptors in the 30 min period following its injection we infused the same amount continuously during 15 min. At first sight, the results seem to support the assumption of rapid dissociation from receptors. However, the decrease in CBF was moderate and it did not affect the structures studied in proportion to the reported density of β -receptor sites (Alexander et al. 1975).

Four facts make us wonder if the moderate effect on CBF observed in the constant-infusion group is at all related to β -receptor inhibition. First, in the cerebellum, hippocampus, and cerebral cortex noradrenaline appears to mediate inhibition of neuronal activity (Segal and Bloom 1976a,b, Hoffer et al. 1973). It seems farfetched to assume, therefore, that β -receptor inhibition should decrease metabolic rate and blood flow. Second, in hypercapnia lesions of the locus coeruleus system fail to alter CBF and CMR_{O_2} (Dahlgren et al. 1981b). Third, in all studies in which propranolol was found to reduce hypercapnic flow rates, CMR_{O_2} was decreased as well (Aoyagi et al. 1976, MacKenzie et al. 1976, Berntman et al. 1979, Hemmingsen et al. 1979). Fourth, if excessive doses of propranolol are given, local glucose consumption is reduced to markedly low values (Savaki et al. 1978). We tentatively conclude, therefore, that when CBF is decreased in hypercapnic animals this

is secondary to a change in metabolic rate. Furthermore, these effects are probably due to an unspecific, local anesthetic effect of high doses of the drug, unrelated to specific effects on β -adrenoceptors.

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Cerebral blood flow and oxygen consumption in normocapnia and
hypercapnia: Modulating influence of paravertebral sympathetic
blockade at the low thoracic level

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ABSTRACT

The objective of the present study was to explore whether the systemic consequences of sympathoadrenal activation influence the cerebral circulatory and metabolic effects of hypercapnia in the rat. To that end, a bilateral blockade of the sympathetic chain was performed at the low thoracic level by paravertebral injection of local anaesthetic. The injection was followed by a reduction in blood pressure and, in comparison with animals injected with local anaesthetic intramuscularly, those with paravertebral blockade showed lower blood and tissue concentrations of glucose and lactate.

Overall ('cortical') CBF and CMR_{O_2} were measured with a $^{133}\text{xenon}$ modification of the Kety-Schmidt technique, and local CBF was estimated autoradiographically with ^{14}C -iodoantipyrine as the diffusible tracer. Paravertebral blockade failed to modify the circulatory response to hypercapnia, nor did it prevent the increase in CMR_{O_2} previously noted in this preparation.

In animals maintained ventilated on 70 per cent N_2O , paravertebral blockade reduced overall CBF by 30 per cent and local CBF by 30-40 per cent, with a suggested but statistically nonsignificant reduction in CMR_{O_2} . In unparalyzed, awake animals the blockade failed to affect local CBF. It is concluded, therefore, that blockade of the sympathetic chain only causes a reduction of CBF in the stressful conditions prevailing in paralyzed and ventilated animals.

Although a number of studies have shown that hypercapnia dissociates cerebral blood flow (CBF) and cerebral metabolic rate for oxygen (CMR_{O_2}) there are several unresolved issues. A few years ago, when available data indicated that hypercapnia increases CBF at an unchanged CMR_{O_2} , the main problem was to characterize the mechanisms responsible for the increase in CBF. Most investigators concerned with this problem have concluded that the dominating cause is the reduction in brain extracellular fluid pH. However, several reports demonstrate that other factors at least exert a modulating influence. For example, it has been documented that the CO_2 responsiveness of the cerebral circulation, defined as the ratio $\Delta CBF/\Delta P_{CO_2}$, is reduced when CMR_{O_2} is depressed, e.g. in deep anaesthesia (Fujishima et al. 1971, see also Edvinsson and MacKenzie 1976, Dahlgren and Siesjö 1981c), others have proposed that a brain stem centre is involved in the response (Shalit et al. 1967), and still others have shown that some drugs, notably propranolol and diazepam, reduce the CO_2 responsiveness (Ayoagi et al. 1976, MacKenzie et al. 1976, Berntman et al. 1979a; however, see also Olesen et al. 1978). Recently, it has been documented that the fatty acid cyclo-oxygenase inhibitor indomethacin dramatically curtails the increase in CBF during hypercapnia (Pickard and MacKenzie 1973, Dahlgren et al. 1981b, Dahlgren and Siesjö 1981c).

During the last few years, controversial reports on the effects of hypercapnia on cerebral metabolic rate have appeared (see Siesjö 1980). The controversy revolves around the problem whether hypercapnia decreases or increases CMR_{O_2} , or leaves it unchanged. Working on phencyclidine-anaesthetized baboons Kliefoth et al. (1979), using the ^{15}O technique of Terpogossian et al. (1970), found that hypercapnia (Pa_{CO_2} about 9.3 kPa) decreased CMR_{O_2} by 30 per cent. In contrast, similar experiments on rats, in which CBF and CMR_{O_2} were measured with a ^{133}Xe modification of the Kety-Schmidt technique, showed that hypercapnia (Pa_{CO_2} about 10 kPa) increased CMR_{O_2} by about 25 per cent (Berntman et al. 1979a). Finally Artru and Michenfelder (1980), using their venous out-flow technique in dogs ventilated on 70 per cent N_2O and <0.1 per cent halothane, a technique which they validated by CBF

measurements using ^{133}Xe clearance, reported an unchanged CMR_{O_2} at comparable PaCO_2 values. It should also be recalled that when Berntman et al. (1979a) pretreated their animals with either propranolol or diazepam, drugs that by themselves did not affect CMR_{O_2} under normocapnic conditions, the CMR_{O_2} was depressed rather than increased during hypercapnia.

Clearly, the effect of hypercapnia on CMR_{O_2} may critically depend on experimental conditions (and/or on the species used). The following facts made us wonder if central catecholaminergic activity exerts a modulating influence: (1) hypercapnia induces an increased rate of hydroxylation of tyrosine in the brain (Carlsson et al. 1977, De Yebenes Prous et al. 1977), (2) both propranolol and diazepam prevent a stress-induced increase in central noradrenergic activity (Taylor and Lavery 1969, Corrodi et al. 1971). However, we have failed to find that lesions of the noradrenergic locus coeruleus neuron system alters the circulatory and metabolic response to hypercapnia (Dahlgren et al. 1981a) nor have we found evidence that the dopamine or 5-HT systems are involved (Dahlgren et al. 1981e).

Since hypercapnia leads to sympathoadrenal activation with release of catecholamines from the adrenal glands (Tenney 1956, Morris and Miller 1962, Nahas et al. 1967, Nahas and Steinsland 1968) we tested the effect of adrenalectomy; this procedure was found not to influence the circulatory and metabolic response to hypercapnia (Berntman et al. 1979a). However, since removal of the adrenal glands interferes with the production of both catecholamines and steroids, and since it should leave release of noradrenaline from peripheral nerves unchanged, we decided to evaluate the effect of a blockade of the paravertebral sympathetic chain at the low thoracic level. As the results will show, this procedure did not modulate the effect of hypercapnia on CBF and CMR_{O_2} . However, paravertebral blockade (PVB - abbreviation used in text) proved to reduce CBF both under normocapnic and hypercapnic conditions, with little effect on CMR_{O_2} . For that reason, the procedures are described in some detail.

MATERIALS AND METHODS

1. Animals

All experiments were performed on male Wistar rats (SPF strain, Møllegaard's Avlslaboratorium, Copenhagen, Denmark). The animals, which weighed 245 to 415 g, had free access to tap water and food pellets (Astra-Ewos, Södertälje, Sweden) until operation. Experiments on paralyzed animals were performed during daytime while those on unparalyzed (awake) animals were performed in the evening in order to minimize ambient noise.

2. Drugs

Bupivacaine $2.5 \text{ mg}\cdot\text{ml}^{-1}$ (Marcain^(R), Astra, Södertälje, Sweden) and d-tubocurarine chloride $3 \text{ mg}\cdot\text{ml}^{-1}$ (Tubocurarine^(R) Vitrum, Stockholm, Sweden) were used as undiluted solutions. The bupivacaine solution was coloured by Evans blue (2 per cent solution, 10-20 μl per ml local anaesthetic). Heparin (Vitrum) was diluted in Krebs-Hensleit solution to give a concentration of $300 \text{ IU}\cdot\text{ml}^{-1}$. ^{14}C -iodoantipyrine and ^{14}C -hexadecane (standard) were obtained from the Radiochemical Centre (Amersham, England). The iodoantipyrine was diluted in ethanol to give an activity of $3.7 \text{ MBq}\cdot\text{ml}^{-1}$. For normocapnic and hypercapnic animals, 0.5 and 0.3 ml of this solution was used, respectively. The alcohol was evaporated in a stream of nitrogen and the substance was dissolved in 1.5 and 0.7 ml of Krebs-Hensleit solution, respectively. ^{133}Xe gas was supplied by AB Atomenergi (Studsvik, Sweden).

3. Operative and sampling techniques

a. Paralyzed animals. The animals were anesthetized with halothane 3-4 per cent in a gas mixture of N_2O and O_2 (70:30). When unresponsive, the animals were tracheotomized and connected to a Starling type respirator delivering about 1 per cent halothane in $\text{N}_2\text{O}/\text{O}_2$ (70:30). Immobilization was achieved by d-tubocurarine $1.5 \text{ mg}\cdot\text{kg}^{-1}$ i.v. Body temperature was controlled and kept close to 37°C by means of external heating. One brachial artery, one femoral artery, and one femoral vein were cannulated to accomplish sampling of arterial (brachial) blood for determination of ^{14}C or ^{133}Xe activity, continuous blood pressure recording, arterial sampling for analyses of blood

gases, pH, glucose, lactate, and pyruvate, as well as of bupivacaine concentrations, and for infusion of donor blood (when ever needed).

In animals used for CBF determination with a modified Kety-Schmidt technique the skull bone was exposed through a small skin incision and a hole, about 0.5 mm in diameter, was drilled 7-8 mm caudal to the bregma. This gave access to the superior sagittal sinus and allowed sampling of cerebrovenous blood. In seven of these animals, two gold-plated screws were applied 11-15 mm apart in each parietal bone. The screws, which just penetrated the external lamina, served as EEG electrodes.

One series of animals was used for measurements of tissue (and CSF) metabolites. In these, a plastic funnel was fitted into a skin incision over the exposed skull bone, allowing freezing of the brain in situ, and the neck muscles were reflected to expose the atlanto-occipital membrane, allowing sampling of cisternal CSF.

When operative procedures had been completed, the animals were allowed a 30 min steady state period before experimental manipulations were induced. These included either PVB (experimental animals) or i.m. injection of the local anaesthetic (control animals); however, some control animals did not receive an i.m. injection (see below). After this heparin was given ($300 \text{ IU} \cdot \text{kg}^{-1}$ i.v.). The animals were then either allowed to breathe the $\text{N}_2\text{O}/\text{O}_2$ mixture or were exposed to hypercapnia for 30 min by admixture of 5-6 per cent CO_2 to the insufflated gas.

b. Unparalyzed animals. Anaesthesia (about 1 per cent halothane in $\text{N}_2\text{O}/\text{O}_2$, 70:30) was delivered via a face mask. A tail artery and vein were cannulated. About 0.1 ml of local anaesthetic was infiltrated at incision sites and the incisions were then carefully sutured. A thermistor probe (Ellab, Copenhagen, Denmark) was inserted in the descending part of the colon and fixed by a suture to the tail. PVB was applied when the animals were still under anaesthesia. The animals were then allowed to recover from the anaesthesia. Some were used for studying behaviour and the remainder for measurements of

l-CBF. For determination of l-CBF the animals were placed in a Perspex cage, constructed to allow measurements without immobilization (for details, see Dahlgren et al. 1981d). When in position, the animals were given heparin ($300 \text{ IU} \cdot \text{kg}^{-1}$) and left to eliminate the anaesthetic gases for 30 min. In the subsequent 45 min period, during which the animals were breathing air, their motility and behaviour were observed, and blood pressure, blood gases, pH, and glucose concentrations measured. At the end of this period, l-CBF was measured. If an animal showed signs of excitation or stress (vigorous movements, rapid blood pressure changes, markedly decreased PaCO_2 values or increasing blood glucose concentrations) during the last 10 min, it was excluded from the material.

4. Methods and analytical procedures.

a. Paravertebral blockade. Bilateral PVB was performed percutaneously. The needle ($0.7 \times 30 \text{ mm}$, Terumo nr.12) was inserted about 20 mm lateral to the spine just caudal to the 13th rib. The needle was advanced cranially at an angle of about 45° to the spine. When the tip of the needle hit the vertebral column it was retracted and then advanced so as to make contact with the lateral-ventral edge of the vertebral body where 0.1 ml of the local anaesthetic was deposited. In anaesthetized animals a successful blockade was judged to be present when mean arterial blood pressure decreased by at least 30 mm Hg within 5 min following the injection. If this requirement was not met after two injections of 0.1 ml each on both sides, the animal was discarded.

After the experiments, the injection sites were examined in all animals. It was then controlled that the (coloured) injectate was deposited around the sympathetic chain, and its diffusion was noted. Animals with gross bleeding intra- or retroperitoneally (or intrapleurally) were discarded, as were those with blue discolouration of the medulla and those that had a satisfactory localization of local anaesthetic on one side only. In unparalyzed animals, which did not have a pre-injection increase in blood pressure (as did paralyzed animals on 70 per cent N_2O) the postmortem examination of the injection sites was the main method for assessing the efficacy

of the blockade. However, as will be described below, a successful blockade was accompanied by typical changes in other variables than the blood pressure.

b. Methods for measuring CBF and CMR_{O_2} . For measurements of ('cortical') CBF and CMR_{O_2} we used a $^{133}\text{xenon}$ modification of the Kety-Schmidt technique with sampling of venous blood from the superior sagittal sinus (Norberg and Siesjö 1974, Berntman et al. 1979b) and of arterial blood from a short brachial catheter (Dahlgren et al. in preparation). Constancy in CBF was controlled by repeated estimation of arteriovenous differences in oxygen content (AVDO_2) before and during desaturation. If two consecutive AVDO_2 values varied by more than 15 per cent, the experiment was discarded. We calculated CBF by the trapezoid rule, using a tissue-to-blood partition coefficient of 0.83, and CMR_{O_2} by multiplying CBF with the mean of at least three AVDO_2 values.

Local CBF (l-CBF) was determined autoradiographically with ^{14}C -iodoantipyrine as the diffusible tracer (Sakurada et al. 1978, for details, see Dahlgren et al. 1981b). In normocapnic animals, the tracer was infused for 45 sec, and in hypercapnic animals for 20 sec, before decapitation. During the infusion, 10-14 arterial samples (about 40 μl each) were collected for scintillation counting on a Rackbeta 1215 (LKB Wallac, Turku, Finland). Counting efficiency was estimated by using ^{14}C -hexadecane as internal standard. The ^{14}C activity in 20 μm thick brain tissue slices, and of a calibrated set of standards, was determined with a MacBeth TD 501 densitometer (aperture 1 mm^2).

c. Analytical techniques. Arterial PO_2 , PCO_2 and pH were determined at 37°C with microelectrodes (Eschweiler and Co, Kiel W. Germany and Radiometer, Copenhagen, Danmark), and temperature-corrected. Changes in blood glucose concentration during the experiments were estimated reflectrometrically (Ames Eye-tone, Miles laboratories, Elkhart, Indiana, USA). However, in 12 experiments, blood was also collected in liquid nitrogen for subsequent enzymatic analyses. For measurements of tissue metabolites, the brain was frozen in situ (Pontén et al. 1973) and subsequently processed for enzymatic, fluorometric analy-

ses of phosphocreatine (PCr), ATP, ADP, AMP and cyclic nucleotides (cAMP and cGMP), as well as of glycogen, glucose, lactate and pyruvate (see Folbergrová et al. 1972 a and b). Cisternal CSF was collected in liquid nitrogen and lactate and pyruvate were analysed with similar techniques. Bupivacaine concentrations were determined by massfragmentography (Caldwell et al. 1977, performed by Astra, Södertälje, Sweden).

5. Calculations

Local CBF was calculated according to Sakurada et al. (1978). Statistical differences between groups were calculated with the Student's t-test for unpaired data.

RESULTS

When performing a PVB of the sympathetic chain our objectives were to eliminate or minimize both the release of catecholamines from the adrenal glands and the 'leakage' of noradrenaline from sympathetic nerve endings, thereby reducing some of the systemic consequences of sympathoadrenal activation. Our working assumption was that if these had any influence on CBF and CMR_{O_2} during hypercapnia, the procedure should prevent this influence. However, we were aware of the fact that ventilation of immobilized rats with 70 per cent N_2O by itself may induce some degree of sympathoadrenal activation. Thus, such animals have a higher than normal mean arterial blood pressure and, at least in fed animals, blood glucose levels increase appreciably with time. For that reason we deemed it necessary to evaluate the effect of PVB on CBF and CMR_{O_2} in normocapnic animals. As the results soon showed, PVB significantly reduced CBF, necessitating evaluation of the effect of the local anaesthetic, if any. Normocapnic control animals were therefore given intramuscular injections of bupivacaine. In the first part of the study, the dose given by i.m. injection was similar to that used for PVB. However, since blood analyses, obtained during the investigation, showed higher concentrations following intramuscular than paravertebral injection, the animals subsequently studied were given a lower dose of bupivacaine (see below).

1. General effects of PVB in paralyzed animals.

As observed by postmortem examination, injection of 0.1 to

0.2 ml of local anaesthetic was followed by diffusion of the solution over a distance extending from about the 9th thoracic to about the 3rd lumbar vertebra. The resulting blockade of the sympathetic chain gave rise to a series of changes, the most conspicuous of which was the decrease in blood pressure. However, mere inspection of the fur disclosed a change in the piloerection capacity since animals given PVB had flat fur, extending from the lower part of the thoracic cage caudally.

Fig.1 illustrates the change in blood pressure following paravertebral injection. In the majority of animals studied, the transient decrease in blood pressure, usually to values of 70-80 mm Hg, was followed by a gradual return of pressure to levels of 100-120 mm Hg. In some animals, the initial decrease in pressure was more marked. In the majority of these, we could prevent the pressure from falling below 80 mm Hg by raising the back end of the operation table by 2-3 cm. In a few animals, 2-4 ml of donor blood had to be given to maintain blood pressure above 100 mm Hg.

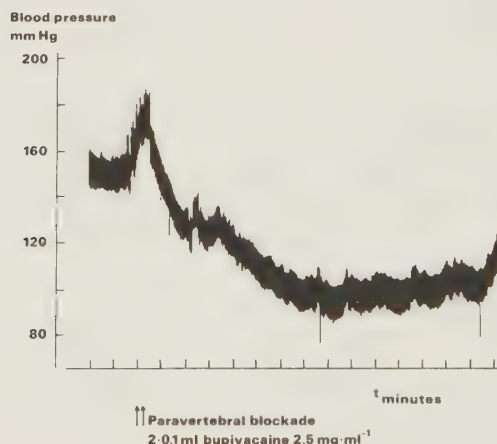


FIG.1. Blood pressure effect of bilateral paravertebral blockade. Application of the blockade indicated by arrows.

Blood pressure was continuously recorded in all animals, and the values measured prior to CBF-CMR_{O2} or I-CBF analyses were those recorded in the tables (see below). In these series, arterial glucose concentrations were measured in 9 normocapnic and 6 hypercapnic animals with PVB. Since the values obtained in the CBF-CMR_{O2} and the I-CBF series were similar, they were pooled for comparisons with control animals in which glucose concentrations were measured. The latter included 11 animals with i.m. injections of bupivacaine and 4 without. The results (Table 1) showed that PVB did not only lower blood

Table 1. Physiological parameters for normo- and hypercapnic animals with and without paravertebral blockade.

Experimental group	Temp °C	MABP mmHg	PaCO ₂ KPa	PaO ₂ KPa	pH	Blood glucose $\mu\text{mol}\cdot\text{ml}^{-1}$
I Normocapnic controls n = 11	37.0 $\pm$ 0.1	140 $\pm$ 3	4.85 $\pm$ 0.09	14.23 $\pm$ 0.13	7.44 $\pm$ 0.01	12.0 $\pm$ 0.8
II Normocapnic with PVB n = 9	37.1 $\pm$ 0.1	117 $\pm$ 5***	5.03 $\pm$ 0.05	14.10 $\pm$ 0.27	7.42 $\pm$ 0.01	8.2 $\pm$ 0.8**
III Hypercapnic controls n = 4	37.0 $\pm$ 0.2	138 $\pm$ 0.1	10.11 $\pm$ 0.27	14.36 $\pm$ 0.27	7.18 $\pm$ 0.01	10.2 $\pm$ 1.2
IV Hypercapnic with PVB n = 6	36.9 $\pm$ 0.1	119 $\pm$ 0.8	10.11 $\pm$ 0.13	14.76 $\pm$ 0.27	7.18 $\pm$ 0.01	6.8 $\pm$ 0.7*

Values are given as means $\pm$ SE

** p<0.01, ***p<0.001 when comparing groups I and II

* p<0.05 when comparing groups III and IV

pressure but also reduced blood glucose concentrations. Physiological parameters (body temperature, PaCO₂, PaO₂ and pH) were similar in PVB and control animals. EEG was continuously recorded in 4 normocapnic and 3 hypercapnic animals. The records failed to reveal any change in EEG pattern following PVB (the records were kindly assessed by Dr. Ingemar Rosén, Department of Clinical Neurophysiology, University of Lund).

A more systematic study of blood glucose concentration was performed in the groups of animals used for analyses of tissue and CSF metabolites (see below). In these, lactate and pyru-

vate concentrations were measured at the same times (15, 30 and 45 min after PVB or i.m. injection of bupivacaine). In one animal with PVB, blood glucose concentrations were grossly aberrant (11.2, 12.5 and 16.2 $\mu\text{mol}\cdot\text{g}^{-1}$, respectively). Since this animal had an initial decrease in blood pressure to 65-70 mm Hg, it was excluded from the material. Fig.2 shows that, in comparison with animals injected with bupivacaine i.m., those given PVB showed consistently lower blood concentrations not only of glucose (cf. Table 1 above) but also of lactate, while differences in pyruvate concentration were significant only at 15 min. PVB also significantly reduced the lactate/pyruvate ratio at 30 min ($p<0.05$) and 45 min ($p<0.01$).

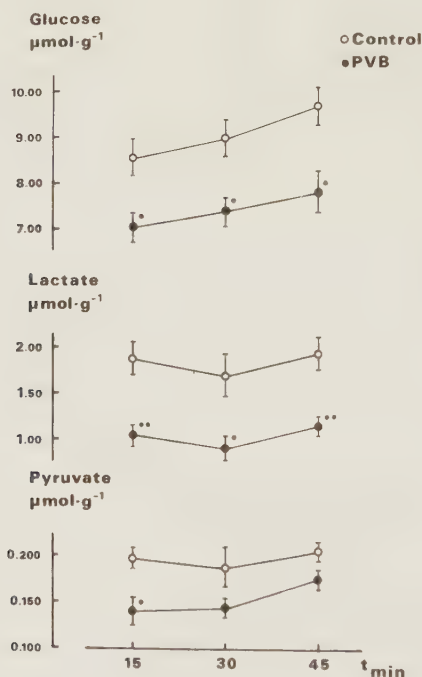


FIG.2. Blood concentration for glucose, lactate and pyruvate (in $\mu\text{mol}\cdot\text{g}^{-1}$) 15, 30 and 45 min after application of local anaesthetic intramuscularly (control) and paravertebrally (PVB). Values are marked as means $\pm$ SEM. * denotes $p<0.05$ and ** $p<0.01$

Since PVB reduced CBF (see below) it seemed of interest to estimate the resorption of local anaesthetic. Blood concentrations of bupivacaine 45 min after injection were measured in 9 animals, 6 of which were given 0.2 and the remainder 0.4 ml of the local anaesthetic. As Fig.3 shows, normocapnic and

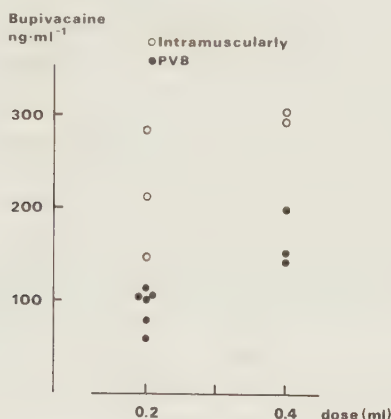


FIG.3. Bupivacaine concentration in blood (in ng·ml⁻¹) 45 min after the injection of 0.2 and 0.4 ml (2.5 mg·ml⁻¹) intramuscularly (circles) and paravertebrally (dots).

hypercapnic animals had blood concentrations of about 100 ng·ml⁻¹ (the values were around 100 and 150 ng·ml⁻¹ with 0.2 and 0.4 ml injectate, respectively). Five analyses on animals given bupivacaine i.m. indicated that, for a similar volume injected, blood concentrations were about twice as high. No pH dependence on blood concentration of bupivacaine was evident. Subsequent control animals were, therefore, given 0.15 ml only. Unfortunately, the samples taken for bupivacaine analyses were lost. However, since the results of Fig.3 indicated that blood concentrations in control animals must have exceeded those in PVB animals, and since bupivacaine i.m. did not seem to affect CBF or CMR_{O₂} (see below) the series were not repeated. Furthermore, since experiments showed that bupivacaine did not affect CBF or CMR_{O₂} at normal or increased CO₂ tensions (cf. also EEG results above), the local anaesthe-

tic was not given to hypercapnic control animals.

2. The effect of PVB on CBF, CMR_{O_2} , and tissue metabolites in normocapnic animals.

Initially, we measured CBF and CMR_{O_2} in 4 normocapnic animals with PVB and compared them with 4 animals given local anaesthetic i.m. Since these studies showed that PVB reduced CBF we extended the series to include 8 animals in each group, performed measurements of local CBF, and analysed tissue and CSF metabolites. We will describe these series in turn.

a. CBF and CMR_{O_2} . Table 2 gives the results obtained in 8 animals with PVB, half of which were given 0.2 ml and the other half 0.4 ml of bupivacaine. Control animals were given either 0.2 (n = 4) or 0.15 ml (n = 4) of the local anaesthetic. Body temperature, PaCO_2 and PaO_2 were similar in the two groups but, in this series, animals with PVB had a slightly lower pH. The blockade decreased mean arterial blood pressure by an average of 30 mm Hg. In the control group, CBF and CMR_{O_2} were similar to previous data obtained under comparable (control)

Table 2. Physiological parameters for normocapnic animals given local anaesthetic intramuscularly or paravertebrally.

Experimental group n = 8	Temp °C	MABP mmHg	PaCO_2 kPa	PaO_2 kPa	pH	$\text{T}\ddot{\text{a}}\text{O}_2$ $\mu\text{mol}\cdot\text{ml}^{-1}$	$\text{AVD}\ddot{\text{O}}_2$ $\mu\text{mol}\cdot\text{ml}^{-1}$	CBF $\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$	CMR_{O_2} $\mu\text{mol}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$
Controls given local anaesthetic intramuscularly	37.1 ± 0.1	145 ± 3	4.87 ± 0.13	14.36 ± 0.27	7.43 ± 0.01	10.41 ± 0.33	4.04 ± 0.28	1.04 ± 0.09	4.02 ± 0.19
PVB	38.8 ± 0.2	116*** ± 4	4.87 ± 0.11	14.10 ± 0.40	7.38** ± 0.01	9.86* ± 0.18	4.91* ± 0.26	0.74* ± 0.04	3.59 ± 0.13

Values are given as means $\pm$ SE

* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$

conditions (see Dahlgren et al. 1981b), indicating that bupivacaine did not alter CBF or CMR_{O_2} from the normal. However, PVB decreased CBF significantly to values that have previously not been recorded under similar conditions. There was a 10 per cent decrease in calculated CMR_{O_2} but this change was not significant ($0.05 < p < 0.10$).

b. Local CBF. Six animals with PVB (bupivacaine 0.2-0.4 ml) were studied. Since it was of interest to compare local CBF values obtained in control animals injected with bupivacaine

i.m. with those measured in uninjected control animals, data from a recent series of experiments were included in the tables. These data were obtained under closely similar experimental conditions, and with an identical l-CBF technique (Dahlgren et al 1981b).

Table 3 shows the physiological data. Animals injected with bupivacaine i.m. (group II) had a slightly lower blood pressure than uninjected control animals (group I); other parameters were similar. Animals given PVB (group III) had a mean arterial blood pressure similar to that of previous series (see Table 1) but, in the present series, the pressure was not significantly different from control (group II). We note that animals with PVB had a slightly higher PaCO_2 .

Table 3. Physiological parameters in normocapnic animals subjected to l-CBF determination. The table comprises values for uninjected controls, animals given local anaesthetic intramuscularly and animals given local anaesthetic paravertebrally.

Experimental group	Temp °C	MABP mmHg	PaCO_2 kPa	PaO_2 kPa	pH
I Controls	37.3 $\pm$ 2	144 $\pm$ 4	5.07 $\pm$ 0.13	13.70 $\pm$ 0.27	7.42 $\pm$ 0.02
II Controls given local anaesthetic intramuscularly n = 5	37.1 $\pm$ 0.2	132 $\pm$ 3 ^x	4.77 $\pm$ 0.07	14.10 $\pm$ 0.40	7.45 $\pm$ 0.01
III Animals given PVB n = 6	37.3 $\pm$ 0.1	117 $\pm$ 7	4.99 $\pm$ 0.08**	14.49 $\pm$ 0.27	7.43 $\pm$ 0.01

Values are given as means $\pm$ SE.

x denotes $p < 0.05$ when comparing groups I and II

** denotes $p < 0.01$ when comparing groups II and III

Results on l-CBF are given in Table 4. A comparison between animals injected with bupivacaine i.m. and uninjected control animals shows that CBF was different in two structures only (septal nuclei and hippocampus). Since this difference was of low significance we conclude that i.m. bupivacaine, in the doses employed, has little or no effect on CBF (cf. similar data in Table 2). In comparison with animals injected with bupivacaine i.m. those given PVB showed a clear decrease in CBF. This decrease affected all structures with relatively little variation (the l-CBF values ranged between 59 and 69 per cent of control). The values obtained in frontal, parietal and sensorimotor cortex, structures that probably drain their blood into the superior sagittal sinus, averaged 65 per

Table 4. 1-CBF values (in $\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$) for normocapnic animals without local anaesthetic, with local anaesthetic intramuscularly, and with local anaesthetic paravertebrally.

Structure	Controls without local anaesthetic n = 6	Animals given local anaesthetic intramuscularly n = 5	Animals given PVB n = 6
Frontal cortex	1.58 ± 0.20	1.69 ± 0.19	0.99 ± 0.08^b
Nc ruber	1.46 ± 0.10	1.42 ± 0.08	0.96 ± 0.3^c
Striatum	1.44 ± 0.09	1.55 ± 0.10	0.97 ± 0.07^c
Subst. nigra	0.99 ± 0.09	1.09 ± 0.04	0.71 ± 0.02^c
Cerebellum	0.92 ± 0.10	1.12 ± 0.04	0.68 ± 0.04^c
Sensory motor cortex	1.69 ± 0.20	1.87 ± 0.22	1.19 ± 0.11^B
Parietal cortex	1.76 ± 0.17	1.72 ± 0.13	1.18 ± 0.10^B
Thalamus	1.33 ± 0.09	1.40 ± 0.09	0.92 ± 0.06^c
Nc ant.thalami	1.41 ± 0.09	1.48 ± 0.09	0.99 ± 0.07^b
Nc vent.post thalami	1.56 ± 0.11	1.66 ± 0.09	1.04 ± 0.07^c
Visual cortex	1.27 ± 0.13	1.33 ± 0.11	0.87 ± 0.08^B
Lat. geniculate body	1.45 ± 0.15	1.43 ± 0.09	0.98 ± 0.04^c
Sup. colliculus	1.56 ± 0.09	1.58 ± 0.06	1.04 ± 0.05^c
Auditory cortex	2.49 ± 0.14	2.52 ± 0.20	1.66 ± 0.10^b
Med. geniculate body	1.79 ± 0.11	1.93 ± 0.10	1.24 ± 0.02^c
Inf. colliculus	1.79 ± 0.13	2.03 ± 0.11	1.21 ± 0.06^c
Sup. olive	1.55 ± 0.09	1.74 ± 0.11	1.06 ± 0.06^c
Hippocampus	0.76 ± 0.04	$0.89 \pm 0.02^*$	0.54 ± 0.02^c
Amygdala	0.88 ± 0.05	1.01 ± 0.04	0.64 ± 0.03^c
Septal nc	0.77 ± 0.04	$0.98 \pm 0.05^*$	0.68 ± 0.05^b
Habenula	1.57 ± 0.11	1.67 ± 0.10	0.99 ± 0.07^c
Hypothalamus	0.93 ± 0.06	0.99 ± 0.04	0.66 ± 0.02^c

Values are given as means $\pm$ SE. *denotes $p < 0.05$ when comparing controls and animals given local anaesthetic intramuscularly; a denotes $p < 0.005$, b denotes $p < 0.01$, and c denotes $p < 0.001$ when comparing the former group with the PVB group.

cent of control (cf. the 30 per cent decrease in CBF in Table 2 above).

c. Metabolites in CSF and cortical tissue. Five animals with PVB received 0.2-0.4 ml bupivacaine (0.4 ml in 4, 0.2 in one). These were compared with 6 animals given local anaesthetic i.m. (0.2 ml in 4, 0.4 ml in 2 animals). At the end of the 45 min period, cisternal CSF was taken for analysis, and the tissue was frozen in situ for analyses on fronto-parietal cortical tissue. Tissue concentrations of PCr, ATP, ADP, and AMP were identical in the two groups. Perhaps more interesting was the fact that PVB did not affect the glycogen concentration (in PVB and control animals, the concentrations were 2.46 ± 0.22 and $2.47 \pm 0.15 \mu\text{mol}\cdot\text{g}^{-1}$, respectively), nor did it change the concentrations of cAMP (1.52 ± 0.04 and $1.47 \pm 0.02 \mu\text{mol}\cdot\text{kg}^{-1}$, respectively) or cGMP (0.032 ± 0.008 and $0.033 \pm 0.006 \mu\text{mol}\cdot\text{kg}^{-1}$, respectively).

In the two groups (PVB and control animals), tissue glucose concentrations were 2.91 ± 0.23 and $3.53 \pm 0.18 \mu\text{mol}\cdot\text{g}^{-1}$ respectively ($p < 0.05$). Since blood glucose concentrations were relatively stable in the 30 min period preceding sampling we used the last blood sample for calculating tissue to blood glucose concentration ratios. The resulting values were 0.36 ± 0.01 and 0.37 ± 0.01 for PVB and controls, respectively. Thus, PVB did not affect glucose distribution between blood and tissue. Since lactate and pyruvate concentrations were measured in blood, CSF and tissue it was possible to calculate intracellular concentrations.

As Table 5 shows, the reduction in blood lactate (and pyruvate) concentrations was not associated with a significant decrease in the CSF or intracellular lactate or pyruvate concentrations. However, the lactate/pyruvate ratio was reduced in cerebral intracellular fluid.

Table 5. Concentrations of lactate and pyruvate in blood, cerebrospinal fluid (CSF), brain tissue and intracellular (i.c.) water 45 min after injection of local anaesthetic intramuscularly or paravertebrally. Table also gives ratio for lactate and pyruvate in CSF and intracellular water. Values are given in $\mu\text{mol}\cdot\text{g}^{-1}$

Experimental group	Blood		CSF		Tissue		Intracellular water		Lact./pyr.	
	lact.	pyr.	lact.	pyr.	lact.	pyr.	lact.	pyr.	CSF	i.c. water
Controls with local anaesthetic intramuscularly n = 6	1.93 ± 0.18	0.205 ± 0.010	2.36 ± 0.14	0.210 ± 0.005	1.53 ± 0.08	0.145 ± 0.006	1.79 ± 0.11	0.172 ± 0.007	11.23 ± 0.58	10.41 ± 0.97
Animals given PVB n = 5	1.14** ± 0.10	0.176 ± 0.009	2.11 ± 0.14	0.193 ± 0.006	1.33 ± 0.08	0.145 ± 0.006	1.49 ± 0.11	0.177 ± 0.011	10.92 ± 0.40	8.42** ± 0.93

Values are given as mean $\pm$ SE.

** $p < 0.01$

In summary, although PVB reduced the blood concentrations of glucose and lactate, and lowered the lactate/pyruvate ratio, the only significant cerebral metabolic effect was a small reduction in tissue glucose concentration and in the intracellular lactate/pyruvate ratio.

3. The influence of PVB on the circulatory and metabolic response to hypercapnia.

We evaluated the effect of hypercapnia, using both CBF-CMR_O₂ and I-CBF measurements. Physiological parameters in PVB and control animals were similar to those given in Table 1 (see above). Thus, in control animals (n = 6 + 6) body temperature,

mean arterial blood pressure, PaCO_2 and pH were $37.0 \pm 0.1^\circ\text{C}$ 143 ± 2 mm Hg, 10.05 ± 0.13 kPa and 7.16 ± 0.01 units, respectively. In PVB animals, the corresponding values were $36.9 \pm 0.1^\circ\text{C}$, 116 ± 0.5 mm Hg, 10.19 ± 0.13 kPa, and 7.18 ± 0.01 units respectively. Thus, whereas all other parameters were similar PVB reduced blood pressure by an average of 27 mm Hg ($p < 0.001$).

a. CBF and CMRO_2 . Fig. 4 shows values for CBF and CMRO_2 . For comparison, the corresponding normocapnic values were included (data from Table 2 above). The following results emerge. First, a comparison between the hypercapnic groups showed that PVB somewhat reduced the mean CBF value, although not significantly ($p < 0.2$). However, since the control CBF was lower, the relative increases in CBF induced by hypercapnia were similar (a-

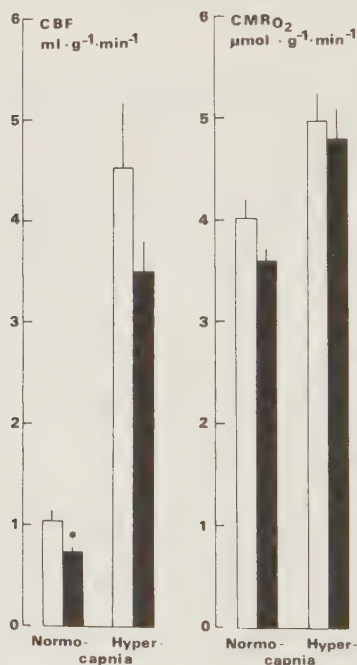


FIG.4 Cerebral blood flow (CBF, in $\text{ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$) and cerebral metabolic rate for oxygen (CMRO_2 in $\mu\text{mol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$) during normocapnia and hypercapnia in animals without (unfilled staples) and with para-vertebral blockade (filled staples). Values are means $\pm$ S.E.M. and *denotes $p < 0.05$.

bout 4.5-fold). In all probability, the suggested reduction of CBF in PVB animals during hypercapnia was due to the reduced blood pressure. Second, PVB did not affect CMR_{O_2} during hypercapnia. In the control group, one aberrant value was obtained ($6.8 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$). If that was excluded, mean values ($\pm$ S.E.M.) for the control and PVB animals were 4.62 ± 0.16 and $4.79 \pm 0.30 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$, respectively. Third, a comparison with normocapnic control values shows that hypercapnia increased CMR_{O_2} ($p < 0.05$ for control groups and $p < 0.01$ for PVB groups; cf. Introduction). We may conclude, therefore, that PVB failed to modulate the CBF response to hypercapnia and prevent the increase in CMR_{O_2} induced by the raised PaCO_2 .

b. Local CBF. Table 6 gives values for l-CBF in hypercapnic animals (control and PVB). In virtually all of the structures analysed, CBF was lower in animals with PVB. However, in ana-

Table 6. l-CBF values (in $\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$) for hypercapnic controls and hypercapnic animals given PVB.

Structure	Control n=6	PVB n=6
Frontal cortex	4.43 ± 0.13	2.55 ± 0.24^c
Nc. ruber	3.93 ± 0.40	2.70 ± 0.16^a
Striatum	4.18 ± 0.25	2.60 ± 0.16^c
Substantia nigra	3.18 ± 0.24	1.96 ± 0.11^c
Cerebellum	2.76 ± 0.22	1.79 ± 0.09^b
Sensory motor cortex	5.65 ± 0.17	2.71 ± 0.27^c
Parietal cortex	4.75 ± 0.21	2.38 ± 0.20^c
Thalamus	4.26 ± 0.20	2.75 ± 0.25^c
Nc. ant. thalami	5.30 ± 0.52	3.48 ± 0.39^a
Nc. vent. post. thalami	5.08 ± 0.22	2.96 ± 0.31^c
Visual cortex	2.68 ± 0.09	1.88 ± 0.14^c
Lat. geniculate body	4.26 ± 0.29	2.77 ± 0.23^b
Sup. colliculus	5.07 ± 0.65	3.11 ± 0.20^a
Auditory cortex	6.03 ± 0.30	3.73 ± 0.34^c
Medial geniculate body	6.08 ± 0.55	3.58 ± 0.27^a
Inf. colliculus	5.80 ± 0.54	3.17 ± 0.19^c
Sup. olive	4.79 ± 0.47	2.97 ± 0.15^b
Hippocampus	2.46 ± 0.19	1.51 ± 0.06^c
Amygdala	2.86 ± 0.24	1.90 ± 0.10^b
Septal nuclei	2.13 ± 0.13	1.46 ± 0.06^b
Habenula	4.94 ± 0.43	3.07 ± 0.25^b
Hypothalamus	2.51 ± 0.25	1.82 ± 0.13^a

Values are given as means $\pm$ SE. a denotes $p < 0.05$, b denotes $p < 0.01$, and c denotes $p < 0.001$

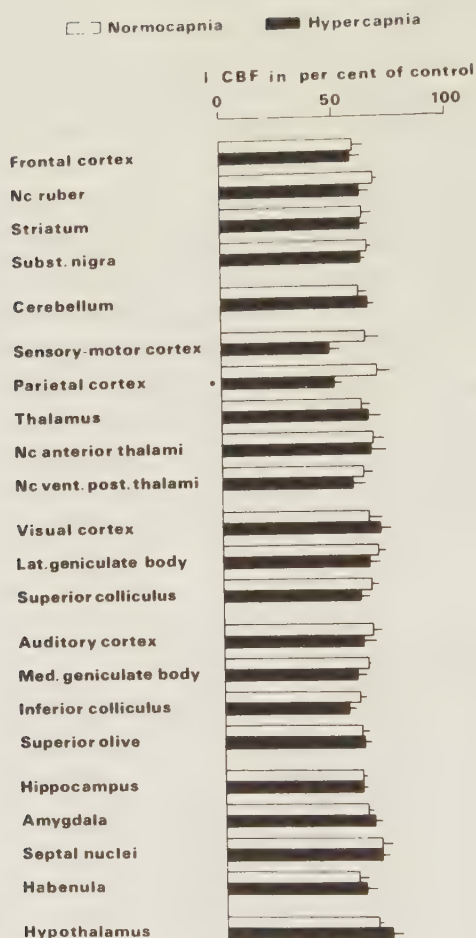


FIG.5 Local cerebral blood flow (l-CBF) for 22 brain structures after paravertebral blockade in normocapnia and hypercapnia. Values are expressed in per cent of respective unblocked controls as means $\pm$ SEM.

*denotes $p < 0.05$

logy with what has been discussed above it is tempting to conclude that this was related to the lower mean arterial blood pressure. This conclusion is supported by results demonstrating that in all but one structure, the percentage reduction

in CBF, induced by PVB, was similar (Fig.5). In other words, the relative increase in CBF was the same in control and PVB animals (cf. data for CBF measured with $^{133}\text{xenon}$).

4. Effects of PVB on unparalyzed animals.

Having established that PVB did not modulate the cerebral circulatory or metabolic response to hypercapnia we returned to the finding that the procedure reduces 'resting' CBF by as much as 30-40 per cent. The question arose whether PVB affects behaviour in unparalyzed animals, and whether it lowers CBF in the absence of nitrous oxide analgesia.

Following the insertion of a tail artery catheter 3 animals were allowed to recover from the anaesthesia. Their behaviour was then studied. The animals showed normal motility, and reacted normally to handling.

Table 7. ^{133}I -CBF values in $\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$ for 22 brain structures in awake animals with and without paravertebral blockade.

Structure	Controls n = 6	Animals with PVB n = 6
Frontal cortex	1.20 $\pm$ 0.05	1.21 $\pm$ 0.05
Nc. ruber	1.53 $\pm$ 0.05	1.45 $\pm$ 0.04
Striatum	1.38 $\pm$ 0.04	1.35 $\pm$ 0.05
Subst.nigra	1.11 $\pm$ 0.05	1.11 $\pm$ 0.04
Cerebellum	1.44 $\pm$ 0.07	1.42 $\pm$ 0.07
Sensory motor cortex	1.46 $\pm$ 0.06	1.40 $\pm$ 0.07
Parietal cortex	1.52 $\pm$ 0.06	1.48 $\pm$ 0.10
Thalamus	1.46 $\pm$ 0.08	1.43 $\pm$ 0.07
Nc. ant. thalami	1.67 $\pm$ 0.04	1.71 $\pm$ 0.06
Nc. vent.post thalami	1.75 $\pm$ 0.09	1.72 $\pm$ 0.09
Visual cortex	1.26 $\pm$ 0.04	1.32 $\pm$ 0.08
Lat. geniculate body	1.44 $\pm$ 0.07	1.42 $\pm$ 0.08
Sup.colliculus	1.75 $\pm$ 0.05	1.61 $\pm$ 0.06
Auditory cortex	2.18 $\pm$ 0.08	1.86 $\pm$ 0.11
Med. geniculate body	1.93 $\pm$ 0.05	1.85 $\pm$ 0.07
Inf. colliculus	3.16 $\pm$ 0.07	3.17 $\pm$ 0.33
Sup. olive	2.31 $\pm$ 0.10	1.76 $\pm$ 0.14
Hippocampus	1.00 $\pm$ 0.03	0.95 $\pm$ 0.04
Amygdala	1.15 $\pm$ 0.09	0.98 $\pm$ 0.04
Septal nc	1.27 $\pm$ 0.07	1.12 $\pm$ 0.02
Habenula	1.75 $\pm$ 0.08	1.76 $\pm$ 0.06
Hypothalamus	1.27 $\pm$ 0.06	1.20 $\pm$ 0.04

Values are given as means $\pm$ SE. No statistical differences between groups.

In six animals, 1-CBF was measured following PVB. Since the procedures used (except for the PVB) were identical to those employed in a previous study on awake, unparalyzed animals (Dahlgren et al. 1981d) no additional control group was studied. The results showed that control and PVB animals had similar values for physiological parameters. For example, mean arterial blood pressure, PaCO_2 and blood glucose concentrations were 111 ± 5 and 104 ± 2 mm Hg, 5.08 ± 0.17 and 5.37 ± 0.07 kPa, and 8.2 ± 0.7 and $7.2 \pm 0.3 \mu\text{mol}\cdot\text{ml}^{-1}$, respectively. Results on 1-CBF are given in Table 7. As observed, in the majority of structures analysed the PVB failed to alter 1-CBF in awake, unparalyzed animals. Thus, the reduction in CBF observed in paralyzed animals seemed to result from an interaction of sympathetic blockade and the anaesthetic procedure (see Discussion).

DISCUSSION

Initially, the objective of the present study was to explore the influence, if any of a paravertebral blockade of the sympathetic chain on the cerebral circulatory and metabolic effects induced by hypercapnia. As the results demonstrate, this procedure failed to alter these responses. The results thus fail to demonstrate that the systemic consequences of peripheral sympathoadrenal activation modifies the CBF response to hypercapnia, nor do they provide hints to the increase in CMR_{O_2} . However, since the results demonstrated that PVB reduces overall and local CBF by as much as 30 per cent (without significantly affecting CMR_{O_2}) we undertook a more extensive study of the physiological and metabolic effects of PVB than originally planned. In doing this, we were inspired by clinical findings demonstrating that sympathetic blockade of about the same thoracic extension ($\text{Th}_{\text{IV-S}_\text{V}}$), through epidural blockade, significantly reduced endocrine and glucose perturbation elicited by abdominal surgery when compared with conditions under conventional anaesthetic procedures, and favourably influenced postoperative catabolism (for summary and further references see Kehlet and Brandt 1980). Before

the effects of PVB are considered it seems warranted to comment on an unrelated issue, i.e. discrepant CBF values obtained with two different techniques.

1. Overall and local CBF

In a previous article (Abdul-Rahman et al. 1979) we obtained autoradiographic values for local CBF that were in good agreement with those measured with the ^{133}Xe modification of the Kety-Schmidt technique; accordingly, we concluded that the two techniques gave concordant results (see also Siesjö et al. 1980). However, a subsequent study revealed that the autoradiographic l-CBF values published were 5-10 per cent too low. In other words, the similarity in CBF values was no longer that apparent. The present study, in which CBF was measured with the two techniques in animals treated similarly (Tables 2 and 4) clearly show that the l-CBF values measured exceed those derived with the ^{133}Xe technique. Since the latter is based on the Fick principle they should represent valid estimates of CBF. It should also be recalled that another CBF technique, applied to rats ventilated on $\text{N}_2\text{O}/\text{O}_2$ has given results very similar to those obtained with the Kety-Schmidt technique presented in this article (Hemmingsen et al. 1979). Possibly, part of the difference noted could be related to the fact that the tissue areas drained by the superior sagittal sinus are not known. Conceivably, some of these include white matter areas, or subcortical grey matter areas with low flow rates. It is also possible that densitometric evaluation of CBF in cortical structures gives a bias toward central layers with high flow rates. Whatever the explanation, some caution should be exercised when overall and local CBF values are compared. In this context, it must also be emphasized that calculation of l-CBF rests on the assumption that equilibration of isotope between blood and tissue is not diffusion-limited (see Eklöf et al. 1974, Eckman et al. 1975). If diffusion-limitation is at hand, l-CBF will be underestimated especially at high flow rates. It is inadvisable therefore, to compare relative changes in CBF, e.g. during hypercapnia, derived with the two different techniques.

In the present experiments, we used a short brachial catheter to sample arterial blood during $^{133}\text{xenon}$ desaturation.

The results indicate that this sampling procedure minimizes any delay in assessing arterial ^{133}Xe activities. Theoretically, this should give more correct (and lower) CBF values at high flow rates. However, calculated CMR_{O_2} values were similar to those reported previously (Berntman et al. 1979a). The present results confirm the previous findings in showing that hypercapnia, at least in the rat, augments CMR_{O_2} (see also Dahlgren and Siesjö 1981c).

2. PVB and inhibition of peripheral sympathetic tone.

In performing the PVB, we aimed at blocking sympathetic tone in abdominal structures thus minimizing release of catecholamines from the adrenal glands and reducing leakage of noradrenaline from sympathetic nerves. However, since the PVB did not affect intrathoracic structures, including the heart, some leakage of noradrenaline must have persisted. In this context, it should be emphasized that the PVB did not affect the cervical sympathetic chain (see above). Apart from the fact that each animal included in the study had verified localizations of injected bupivacaine around the sympathetic chains, the following findings attest to the efficacy of the injections; (1) blood pressure fell, initially by as much as 30 to 50 mm Hg, (2) blood glucose concentrations were maintained at levels considered normal, and (3) in comparison to control animals those with a successful PVB showed reduced blood lactate concentrations and lactate/pyruvate ratios. In all probability, these effects reflect reduced peripheral α -adrenergic constrictory tone and reduced catecholamine-mediated glycogenolysis in the liver. Admittedly, analyses of plasma catecholamine levels would have been desirable. We deemed it warranted, though, to report the results without such information, and to postpone analyses of catecholamines until we have access to suitable micromethods.

3. PVB and cerebral blood flow.

There is little information on the effect of a blockade of the lower part of the sympathetic chain on CBF (or CMR_{O_2}). Kety et al. (1950) reported a fall of about 10 per cent in CBF when blood pressure fell by an average of 50 mm Hg subsequent to a differential spinal sympathetic block with exten-

sion up to the mid-thoracic region achieved by procaine infusion into the lumbar subarachnoidal space. However, apart from the fact that this small effect on CBF was obtained with a much more marked lowering of blood pressure, the results were obtained on hypertensive patients who are known to have a diminished ability to autoregulate. Galindo (1964) reported a 16 per cent reduction in internal carotid blood flow in the dog after total epidural anesthesia. The hypercapnic response was markedly curtailed by the blockade. However, changes in internal carotid blood flow do not reflect circulatory events in the brain (see Harper et al. 1977). Sivarajan et al. (1976) studied the effects of sympathectomy by epidural blockade at two thoracic levels (Th_X and Th_I), using a microsphere technique in the dog. Their results showed that a blockade of the total thoracic region reduced CBF by up to 35 per cent, a reduction which was attributed to abolished autoregulation. It seems highly unlikely that loss of autoregulation could explain the present results, especially since l-CBF fell by 30 to 40 per cent in animals showing a reduction in blood pressure by less than 20 mm Hg (see also Harper et al. 1977).

Crucial to the present discussion is the effect of PVB on l-CBF in awake, unrestrained animals not exposed to 70 per cent N_2O . Thus, the question had to be posed whether PVB reduces a dilatory tone related to immobilization and N_2O administration. What argues against this possibility is the fact that in paralyzed animals breathing N_2O , PVB reduced CBF to values that are considerably lower than those obtained in awake, partially restrained animals (Sakurada et al. 1978, Dahlgren et al. 1981d). However, as the results on awake animals demonstrate, PVB was without effect on l-CBF. We are then left with the observation that PVB markedly reduces CBF only with a background of N_2O analgesia and immobilization. At present any explanation must be speculative. However, in view of the widespread clinical use of N_2O , and of the potential benefits of a spinal sympathetic block, the mechanisms involved warrant further study.

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The effect of indomethacin on cerebral blood flow and oxygen consumption in the rat at normal and increased carbon dioxide tensions

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ABSTRACT

The effect of the fatty acid cyclo-oxygenase inhibitor indomethacin on cerebral blood flow (CBF) and the metabolic rate for oxygen (CMR_{O_2}) was studied in paralyzed and artificially ventilated rats. In normocapnic animals, the drug ($10 \text{ mg}\cdot\text{kg}^{-1}$ i.v.) reduced CBF to 50% of control without a measurable effect on CMR_{O_2} . During hypercapnia ($PaCO_2$ 70-80 mm Hg) the increase in CBF was reduced by about 80% but CMR_{O_2} remained unchanged. Autoradiographic evaluation of local CBF in 20 brain structures indicated that the reduction in CBF was relatively uniform throughout the brain. Dose response curves showed that an effect on CBF was evident already at an indomethacin dose of $1 \text{ mg}\cdot\text{kg}^{-1}$ and maximal effects were obtained with $3\text{-}5 \text{ mg}\cdot\text{kg}^{-1}$. Following i.v. injection of the drug reduction in CBF was observed already after 10 s and the full response occurred after 1-2 min. It is concluded that metabolites of arachidonic acid, possibly mainly prostacyclin, are powerful modulators of normal cerebrovascular tone, and help to mediate the CBF response to increased CO_2 tensions. However, since indomethacin does not modify the circulatory response in other conditions with increased CBF these substances do not qualify as general coupling factors controlling CBF in physiological or pathological states.

INTRODUCTION

Recent results demonstrate that the fatty acid cyclo-oxygenase inhibitor indomethacin markedly reduces cerebral blood flow (CBF) without a corresponding influence on the metabolic rate for oxygen (CMR_{O_2}). In phencyclidine-anaesthetized baboons, Pickard & MacKenzie (1973) found that intravenous or intracarotid infusion of indomethacin ($10 \text{ mg}\cdot\text{kg}^{-1}$ and $0.04\text{-}0.2 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, respectively) reduced CBF by 38% at normocapnia, with no significant effect on CMR_{O_2} . Indomethacin also markedly attenuated the increase in CBF during hypercapnia. Subsequent results from the same laboratory (Pickard et al. 1977b) showed that following administration of indomethacin, the cerebral vessels retained their ability to autoregulate when the perfusion pressure was varied. Thus, if one assumes that indomethacin in the doses used predominantly inhibits the fatty acid cyclo-oxygenase (see Flower 1974) the results suggest that

some unidentified prostaglandins help to maintain cerebrovascular tone during normocapnia and mediate the increase in CBF during hypercapnia, but that they are not responsible for autoregulation.

Results on rats reported in preliminary form from this laboratory confirmed the influence of indomethacin on CBF at normocapnia and hypercapnia (Sakabe & Siesjö 1979). In fact, in this study CBF was reduced to 50% of control in normocapnia. The results also demonstrated that indomethacin was without effect on the cerebral circulatory (and metabolic) response to induced hypoxia.

In this article we give a full account of the experiments designed to study the effect of indomethacin on CBF and CMR_{O_2} at normocapnia and hypercapnia. In addition we provide data on the effect on local CBF (l-CBF) in normo- and hypercapnia. Finally, we further describe effects of indomethacin upon CBF in terms of the dose-response and the time course of response.

MATERIALS AND METHODS

A. Drugs

Indomethacin (Confortid^(R), Dumex) was prepared in 5 or 10 $\text{mg}\cdot\text{ml}^{-1}$ solutions with sterile water for i.v. injection.

B. Operative and sampling techniques.

Experimental groups

All experiments were performed on male Wistar rats of a S.P.F. strain (Møllegaards Avlslaboratorium, Copenhagen) that had free access to rat pellets (Sanbolagen, Malmö) and tap water until operation. Operative and sampling procedures can be described under four headings.

1) Physiological parameters and behaviour in unanesthetized and spontaneously breathing animals. In 2 animals tail artery and vein catheters were inserted under anesthesia with 2-3% halothane. When the animals had fully recovered from the anesthesia their behavior was noted and blood pressure, arterial P_{O_2} , PCO_2 , and pH were measured. Observations were continued following injection of indomethacin in a dose of 10 $\text{mg}\cdot\text{kg}^{-1}$ i.v.

2) Cerebral (cortical) blood flow and oxygen consumption.

Under anesthesia with 2-3% halothane these animals were tracheotomized, immobilized with d-tubocurarine chloride ($1.5 \text{ mg} \cdot \text{kg}^{-1}$) and connected to a Starling type respirator delivering about 1% halothane, 75% N_2O and 25% O_2 . Catheters were placed in the femoral arteries for blood pressure recording and sampling of arterial blood, and in one femoral vein for injections and infusion of donor blood. The caudal part of the superior sagittal sinus was exposed to allow repeated sampling of cerebral venous blood for measurements of (cortical) CBF and CMR_{O_2} . At the end of the operative procedures halothane was discontinued and in the majority of animals ventilation was continued on 75% N_2O and O_2 . Two experimental groups were studied. In the normocapnic group, following a steady state period of about 30 min, indomethacin was injected i.v. at a dose of $10 \text{ mg} \cdot \text{kg}^{-1}$ and the CBF- CMR_{O_2} couple was measured 30 min later. To exclude an interaction between indomethacin and nitrous oxide 4 animals were studied after discontinuation of nitrous oxide supply for 15 min. These animals were adrenalectomized, pain and discomfort were minimized by injection of a local anesthetic into wound sites, and visual and auditory stimuli were excluded (see Carlsson et al. 1977). The hypercapnic animals were treated as those maintained on 70% N_2O except that hypercapnia was induced either 5 min after the injection of indomethacin, CBF- CMR_{O_2} being measured 25 min later, or 15 min after injection, by adding 5% CO_2 to the gas mixture. In the latter group CBF- CMR_{O_2} were measured 30 min later. In two control groups, i.e. normocapnic and hypercapnic animals not given indomethacin, a similar time schedule was followed.

3) Local cerebral blood flow. 2 series of animals were studied, normocapnic and hypercapnic, with and without injection of indomethacin ($10 \text{ mg} \cdot \text{kg}^{-1}$) 30 min prior to measurement of l-CBF. Operative techniques were the same as those described in the preceeding paragraph (2) except that no burr hole was made in the skull, and that one catheter was cut to an extravascular length of 1.5-2 cm to avoid distortion ("smearing") of the curve describing the rate of rise of ^{14}C -iodoantipyrine activity in arterial blood (see below). Hypercapnia, induced as

described above, was maintained for 30 min before 1-CBF was measured.

4) Time and dose dependent changes in overall cerebral blood flow. Animals were anesthetized as under 2) and provided with femoral artery and vein catheters for blood pressure monitoring, arterial blood sampling and i.v. injections. To allow continuous recording of CBF both retroglennoid veins were exposed under halothane- N_2O anesthesia. One vein was cannulated and the cerebral venous outflow was continuously measured with a drop recorder in a closed extracorporeal circuit returning the blood by spontaneous flow to the superior caval vein. During the measurement the contralateral retroglennoid vein was compressed (Nilsson & Siesjö 1980). The time course and dose dependence of the CBF was studied following administration of different doses of indomethacin ($0.5\text{--}10 \text{ mg}\cdot\text{kg}^{-1}$) at normo- and hypercapnia.

C. Methods and analytical techniques

Arterial PCO_2 , PO_2 and pH were measured with microelectrodes at 37°C , with due corrections for any deviation in body temperature. Total oxygen content in arterial and venous blood (CaO_2 and CvO_2) were measured polarographically (Borgström et al. 1974).

Cortical CBF was measured with a $^{133}\text{xenon}$ modification of the Kety-Schmidt technique (Norberg & Siesjö 1974, Berntman et al. 1979a). CMR_{O_2} was calculated from CBF and arteriovenous differences in CO_2 (AVD_{O_2}), the latter representing the average of at least 3 consecutive coupled samples of arterial and cerebral venous blood.

Local CBF was measured with the autoradiographic ^{14}C -iodoantipyrine technique described by Sakurada et al. (1978). Details of procedure have been given in a previous communication (Abdul-Rahman et al. 1979). Due to a slight error in the standardization procedures our previous CBF values were about 5% too high. That error was corrected in the present material. In noninjected hypercapnic animals 1-CBF values were so high that the standard 1-CBF technique was considered inaccurate. In order to minimize distortion of the arterial ^{14}C -activity curve these animals were therefore provided with a short

(1.5-2 cm) catheter in one subclavian artery, and the interval between start of infusion of the tracer and decapitation was reduced to 20 sec (Dahlgren et al., in preparation).

Time dependent changes in CBF were estimated by the technique previously described from the laboratory (Nilsson 1974, Nilsson & Siesjö 1980, see also Meldrum & Nilsson 1976).

D. Calculations

Cortical and local CBF were calculated as described in the articles quoted. Statistical differences were calculated by Student's t-test or the Mann-Whitney U-test. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

RESULTS

1. Behavior

Indomethacin in a dose of $10 \text{ mg} \cdot \text{kg}^{-1}$ i.v. had minor effects on behavior and physiological parameters in freely moving animals. In the 30 min period following injection, both animals studied showed somewhat decreased spontaneous motility but reacted normally to handling and to auditory and painful stimuli. Mean blood pressure remained at values of 100-120 mm Hg and arterial PO_2 was in the normal range (75-90 mm Hg). In both animals PaCO_2 decreased by 5-8 mm Hg but arterial pH remained around 7.4.

2. CBF and CMR_{O_2}

(a) Normocapnia. Table 1 gives the physiological parameters as

TABLE 1. Physiological parameters and $\text{CBF}/\text{CMR}_{\text{O}_2}$ in normocapnic animals: influence of indomethacin $10 \text{ mg} \cdot \text{kg}^{-1}$ i.v.

Experimental groups	Temp °C	MAP mm Hg	PaO_2 mm Hg	PaCO_2 mm Hg	Arterial pH	CaO_2 $\mu\text{mol} \cdot \text{ml}^{-1}$	AVDO_2 $\mu\text{mol} \cdot \text{ml}^{-1}$	CBF $\text{ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$	CMR_{O_2} $\mu\text{mol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$
Control n = 7	37.1 ± 0.1	149 ± 4	108 ± 3	34.8 ± 0.8	7.39 ± 0.01	10.63 ± 0.17	3.66 ± 0.22	1.10 ± 0.11	3.91 ± 0.28
Indomethacin $\text{N}_2\text{O}/\text{O}_2 = 3/1$ n = 6	37.1 ± 0.1	139 ± 5	110 ± 3	37.6 ^x ± 0.9	7.40 ± 0.01	10.72 ± 0.17	6.44 ^{xx} ± 0.33	0.53 ^{xxx} ± 0.03	3.36 ± 0.23
Indomethacin $\text{N}_2/\text{O}_2 = 3/1$ n = 5	37.2 ± 0.1	140 ± 6	117 ± 3	36.0 ± 1.3	7.39 [*] ± 0.01	11.10 ± 0.37	7.15 ^{xx} ± 0.51	0.58 ^{xxx} ± 0.03	4.06 ± 0.22

Mean $\pm$ S.E.M.

well as the cortical CBF, AVD_{O_2} and CMR_{O_2} values measured in normocapnic animals maintained on 75% N_2O or 75% N_2 with 25% O_2 . Body temperature and mean arterial blood pressure (MABP), as well as arterial P_{CO_2} , P_{O_2} , and pH were similar in control and indomethacin-injected animals. In animals maintained on N_2O CBF was reduced to 50% of control and AVD_{O_2} was virtually doubled. As a result, calculated CMR_{O_2} did not change significantly from control. Results obtained in animals in which the nitrous oxide supply was discontinued for 15 min showed a similar decrease in CBF, demonstrating that a pronounced reduction in CBF was not due to an interaction between indomethacin and nitrous oxide. The CMR_{O_2} values were not different from those of the control group.

(b) Hypercapnia. Table 2 gives the physiological parameters and CBF/ CMR_{O_2} in hypercapnic control and indomethacin-injected animals. A comparison with Table 1 shows that hypercapnia increased CBF about 5-fold and, as in a previous study (Berntman et al. 1979b) there was a slight, however in this material not significant, increase in CMR_{O_2} . Indomethacin dramatically reduced the increase in CBF during hypercapnia. In 7 of the animals, indomethacin was given 5 min before induction of hypercapnia and in 4 animals 15 min before induction of hypercapnia. The period of hypercapnia was 25 and 30 min, respectively. The effects on CBF were similar in the two groups. No significant

Table 2. Physiological parameters and CBF/ CMR_{O_2} in hypercapnic animals: influence of indomethacin 10 mg.kg⁻¹ 15 and 5 min prior to hypercapnia 30 min.

Experimental group	Temp °C	MABP mm Hg	P_{aO_2} mm Hg	P_{aCO_2} mm Hg	Arterial pH	Ca_{O_2} $\mu\text{mol}\cdot\text{ml}^{-1}$	AVD_{O_2} $\mu\text{mol}\cdot\text{ml}^{-1}$	CBF $\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$	CMR_{O_2} $\mu\text{mol}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$
Control n=8	36.7 ± 0.1	154 ± 5	121 ± 2	84 ± 2	7.13 ± 0.01	9.91 ± 0.33	0.85 ± 0.07	5.43 ± 0.51	4.52 ± 0.36
Indomethacin 15 min n=7	36.7 ± 0.2	157 ± 4	119 ± 3	77 ^x ± 1	7.17 ± 0.01	10.59 ^x ± 0.21	2.81 ^{xxx} ± 0.3	1.34 ^{xxx} ± 0.12	3.66 ± 0.22
Indomethacin 5 min n=4	37.3 ^{xx} ± 0.2	154 ± 3	120 ± 3	76 ± 1	7.16 ± 0.01	10.37 ± 0.23	2.68 ^{xx} ± 0.4	1.64 ^{xx} ± 0.38	3.91 ± 0.43

Mean $\pm$ S.E.M.

MABP = mean arterial blood pressure

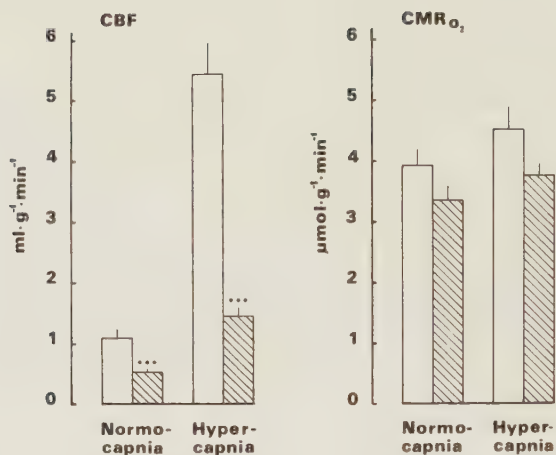


Fig.1. Cerebral blood flow (CBF) and oxygen consumption (CMR_{O₂}) in normocapnic and hypercapnic rats. Open columns: Non-treated animals. Hatched columns: Indomethacin (10 mg.kg⁻¹)-pre-treated animals. Mean $\pm$ S.E.

effect on CMR_{O₂} was found. Fig. 1 which summarizes the CBF/CMR_{O₂} data on Tables 1 and 2, illustrates the following points. First, indomethacin markedly curtailed the increase in CBF during hypercapnia. Thus if the comparison is made to uninjected normocapnic controls indomethacin reduced the 5-fold increase in CBF to 25%. As a result, CBF levels less than 50% above the normocapnic control values were seen. However, if the comparison is made to indomethacin-injected normocapnic animals, hypercapnia was found to increase CBF 2.5- to 3-fold. In other words, relative changes in CBF during hypercapnia was not markedly affected by indomethacin. Second, the changes in absolute flow rates induced by indomethacin in normocapnic and hypercapnic animals occurred without a significant change in CMR_{O₂}. However, the results suggest that in indomethacin-injected animals, hypercapnia may not increase CMR_{O₂} (see above).

3. Local CBF

(a) Normocapnia. In animals used for measurements of l-CBF physiological parameters were similar to those presented in

Table 3. Local cerebral blood flow (l-CBF) in 21 brain structures in normocapnic controls and in normocapnic indomethacin-injected (10 mg·kg⁻¹) animals. Values are given in ml·g⁻¹·min⁻¹ and in per cent of control.

Regions	Normocapnia Control n=6	Normocapnia Indomethacin n=4	Normocapnia Indomethacin Per cent of control
Frontal cortex	1.58 ±0.15	0.62 ±0.04	39 ±3
Nc ruber	1.46 ±0.10	0.76 ±0.03	52 ±2
Striatum	1.44 ±0.09	0.67 ±0.05	51 ±3
Substantia nigra	0.99 ±0.09	0.57 ±0.03	58 ±3
Cerebellum	0.99 ±0.10	0.61 ±0.03	66 ±3
Sensory-motor cortex	1.69 ±0.20	0.77 ±0.10	46 ±6
Parietal cortex	1.76 ±0.17	0.77 ±0.10	43 ±6
Thalamus	1.36 ±0.09	0.81 ±0.09	60 ±7
Nc post. vent. thalami	1.54 ±0.10	0.84 ±0.10	55 ±6
Visual cortex	1.27 ±0.13	0.62 ±0.02	49 ±2
Lat.geniculate body	1.45 ±0.15	0.74 ±0.07	51 ±5
Sup. colliculus	1.56 ±0.09	0.83 ±0.07	53 ±4
Auditory cortex	2.49 ±0.14	0.94 ±0.15	38 ±6
Med.geniculate body	1.79 ±0.11	0.90 ±0.10	50 ±6
Inf. colliculus	1.79 ±0.13	1.25 ±0.18	70 ±10
Hippocampus	0.76 ±0.04	0.46 ±0.03	61 ±4
Amygdala	0.88 ±0.05	0.46 ±0.06	52 ±7
Septal nc	0.77 ±0.04	0.45 ±0.04	58 ±5
Habenula	1.57 ±0.11	0.87 ±0.09	55 ±6
Hypothalamus	0.93 ±0.06	0.50 ±0.02	54 ±2

Mean ± S.E.M.

Table 1. Values for blood pressure in control (uninjected) and indomethacin injected animals were 143±6 and 140±2 mmHg, respectively and for PaCO₂ 38.8±1 and 36.8±2 mmHg, respectively. l-CBF values for 20 different structures are given in Table 3. The results show that indomethacin reduced CBF in all structures analyzed. As shown in Fig. 2 the results amply confirm those with the ¹³³xenon technique. Thus, despite some variation between different structures, the general reduction of l-CBF was in the order of about 50%.

(b) Hypercapnia. Physiological variables of these animals were similar to those described in Table 2. Thus, in uninjected animals PaCO₂ varied between 75 and 79 mmHg, and in indomethacin-injected animals between 70 and 82 mmHg. In order to illustrate that changes in l-CBF during hypercapnia, induced by indomethacin, were similar to those observed at normal CO₂ tensions, the l-CBF values for normocapnia and hypercapnic animals have been given in per cent of their respective controls in Fig. 2.

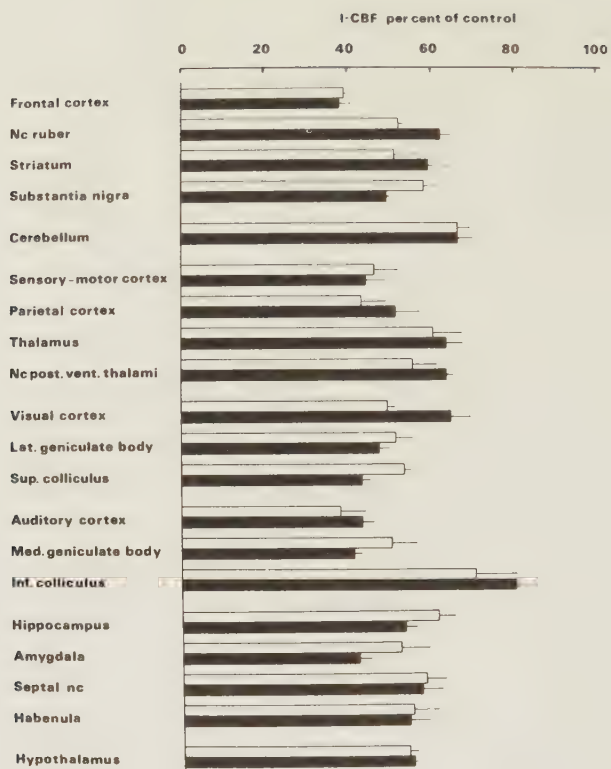


Fig. 2. Relative local cerebral blood flow (l-CBF) in indomethacin-treated normocapnic (open columns) and hypercapnic (filled columns) animals. The values are in per cent of control (mean $\pm$ S.E.). Values for normocapnic animals are those given in Table 3. Values for hypercapnic animals were derived from a group of four indomethacin-injected animals that were compared to a group of 5 uninjected hypercapnic controls.

4. Time course of CBF response

Using the continuous cerebral venous outflow method it was seen that a definite reduction of CBF started about 10 s after an i.v. bolus injection of indomethacin. The response reached near maximal values between 1 and 2 min after injection. Fig. 3 shows an original recording of blood pressure and cerebral venous outflow (drop recording) illustrating in sequence the

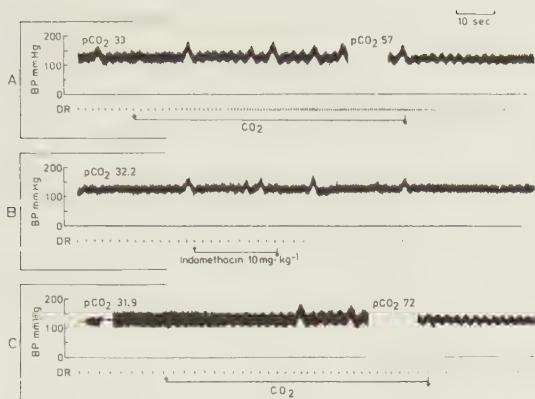


Fig. 3. Original recording (BP = blood pressure, DR = cerebral venous outflow drop recording) from an animal exposed to (in succession) A: Short CO₂ inhalation, B: Indomethacin injection in normocapnia, C: Short CO₂ inhalation after indomethacin injection.

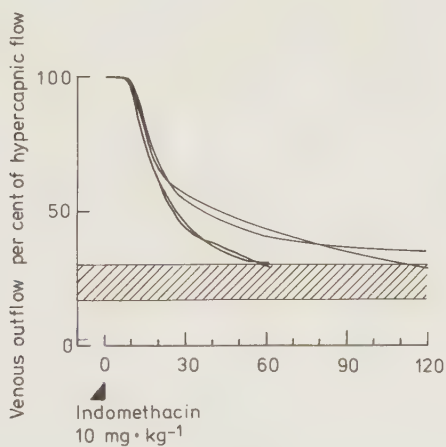


Fig. 4. Time course of CBF response to indomethacin injection (10 mg·kg⁻¹ i.v.) in 4 animals. 100% denotes steady state hypercapnic flow before injection, hatched area normocapnic flow (range) before induction of hypercapnia.

normal flow, the CO₂ response, the indomethacin response (10 mg·kg⁻¹) in the normal flow situation and the CO₂ response after indomethacin injection. Since the range of the flow reduction was more prominent in the hypercapnic animals, a fairly exact timing of the response could be settled in this situation. Fig. 4 shows the effect of indomethacin 10 mg·kg⁻¹ i.v. given as a bolus in a hypercapnic high flow situation in 4 animals. Flow values slightly above normocapnic control values were reached within 1 min. Blood pressure was constant during the period of measurement in these experiments.

5. Dose response of CBF

In order to increase the resolution of the flow method, a systematic dose response study was performed in animals in which a hypercapnic high flow was primarily induced (see above). Fig. 5 shows the effect of accumulated doses after i.v. bolus injection of indomethacin given at about 3 min intervals in 4 animals. It is seen that the maximal response was reached with indomethacin doses of 3-5 mg·kg⁻¹. When the dose response data are given as per cent inhibition of the

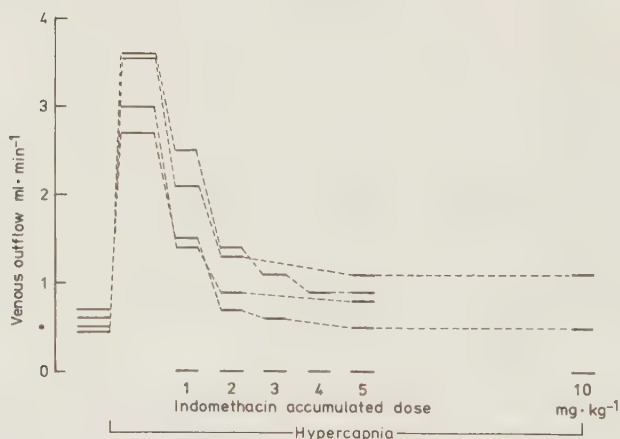


Fig. 5. CBF response to increasing doses of indomethacin i.v. in 4 animals. Start = Normocapnic flow. Hypercapnia (PCO₂ 70-90 mmHg) was then induced and after 4-6 min of stable high flow indomethacin was injected i.v. at 3 min intervals in accumulating doses as indicated on the abscissa.

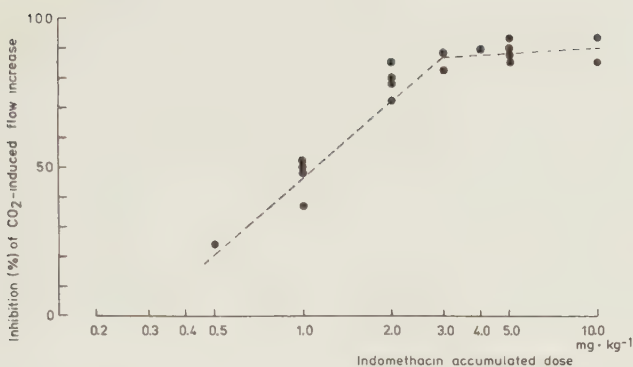


Fig. 6. Dose related inhibition by indomethacin i.v. of calculated CO₂ induced CBF increase. (Data from expts. shown in Fig. 5). The broken line indicates the dose related inhibition of prostaglandin synthesis in rat brain (from Abdel-Halim et al. 1978).

total CO₂ induced CBF increase, a typical dose response curve is obtained. In Fig. 6 the inhibition of CO₂-induced CBF increase is compared with the dose-dependent inhibition of prostaglandin synthesis in the rat brain as described by Abdel-Halim et al. (1978). An excellent correspondence between the two effects of indomethacin is seen. The study also shows that the indomethacin dose of 10 mg.kg⁻¹, used in the CBF/CMR_{O₂} and I-CBF studies, should have been associated with a maximal response.

DISCUSSION

Like other tissues, the brain forms prostaglandins and related substances in response to appropriate stimuli (Samuelsson 1964, Wolfe et al. 1976a and b, Abdel-Halim & Ånggård 1979, Steinhauer et al. 1979). Substances identified include not only PGE₂, PGF_{2α} and PGD₂ but also prostacyclin (Abdel-Halim et al. 1980) and thromboxane A₂ (Steinhauer et al. 1979, see also Marion & Wolfe 1978). The precursor of these substances is arachidonic acid mainly derived from phospholipids with phosphatidyl inositol and phosphatidyl choline as the main sources

(Marion & Wolfe 1978). As in other tissues, prostaglandin formation in the brain tissues is inhibited by indomethacin (Wolfe et al. 1976a and b, Abdel-Halim et al. 1978).

The nature of the prostaglandin-like substances formed seems to differ both between species and between tissue structures. The brain of the rat forms both PGE_2 and $\text{PGF}_2\alpha$ but the major prostaglandin seems to be PGD_2 (Abdel-Halim & Ånggård 1979). It has now been established that vascular tissue preferentially forms prostacyclin (Gryglewski et al. 1976, Moncada et al. 1976) and this has been confirmed for brain vessels (Abdel-Halim et al. 1980).

Formation of prostaglandins and related substances has usually only been observed under pathological conditions, presumably those that are associated with activation of phospholipase A_2 and with accumulation of arachidonic acid. These conditions include in vitro incubation or disintegration (e.g. homogenisation) of tissue (e.g. Wolfe et al. 1976a and b, Abdel-Halim & Ånggård 1979), induction of epileptic seizures (Folco et al. 1977, Marion & Wolfe 1978, Steinhauer et al. 1979), and induction of transient ischemia (Gaudet & Levine 1979). Less is known about the physiological roles of arachidonate metabolites. Prostaglandins of the E type have been reported to have depressant and sedative actions (Horton 1972, 1974). Experiments on baboons have shown that both PGE_2 and $\text{PGF}_2\alpha$, when given by intracarotid infusion with or without prior disruption of the blood-brain barrier, reduce CMR_{O_2} and CBF (Pickard et al. 1977a).

The demonstration by Pickard & MacKenzie (1973) that indomethacin reduces CBF under control conditions, and markedly curtails the circulatory response to hypercapnia, suggests that prostaglandin-like substances regulate cerebrovascular resistance. However, since indomethacin has no effect on the circulatory response to hypoxia such substances cannot be generally responsible for vascular dilatation in the brain (Sakabe & Siesjö 1979). The nature of the substance(s) involved has not been clarified. However, the fact that prostacyclin is a potent vasodilator in the coronary circulation (Dusting et al. 1977, Needleman et al. 1978, Schrör & Moncada 1979) suggests that indomethacin may exert its effect in the brain by blocking

prostacyclin synthesis in the vessel walls. This suggestion is supported by results showing that indomethacin markedly reduces CBF without any effect on CMR_{O_2} (Pickard & MacKenzie 1973, Sakabe & Siesjö 1979, and present results). The results of the present experiments can be summarized as follows. (1) Under control conditions indomethacin in a dose of $10 \text{ mg} \cdot \text{kg}^{-1}$ reduces cortical CBF to 50% of control without any measurable effect on CMR_{O_2} . (2) In hypercapnia the circulatory response is curtailed to about 25% of that observed in uninjected animals but CMR_{O_2} is unaffected. Autoradiographic estimation of I-CBF confirms the $^{133}\text{xenon}$ data and demonstrate a fairly homogenous reduction in I-CBF under both normocapnic and hypercapnic conditions. (3) Dose response curves show that maximal effect on CBF is obtained with a dose of $3\text{--}5 \text{ mg} \cdot \text{kg}^{-1}$. (4) The effect of i.v. indomethacin on CBF is observed already after 10 s, and the maximal effect is attained in 1-2 min. We will discuss these 4 points in turn, and then (5) shortly discuss the significance these findings may have on our concepts of cerebral vasomotor control mechanisms.

1. CBF and CMR_{O_2} under control conditions

The 50% reduction in CBF in normocapnic (and normoxic) rats observed presently exceeds that obtained by Pickard & MacKenzie (1973) in the baboon. At normal blood pressures, a comparable reduction in CBF has only been observed in pronounced hypocapnia (for data on rats, see Eklöf et al. 1973). At normal metabolic rate, this degree of reduction is usually considered to be the maximally tolerable one. However, we have failed to observe changes in the cerebral energy (and redox) state in animals given indomethacin (unpublished results) and tentatively conclude that the energy homeostasis is maintained. Accordingly, the cerebral metabolic changes observed in pronounced hypocapnia (see MacMillan & Siesjö 1973) must at least partly be due to the associated alkalosis.

As stated, the uncoupling of CBF and CMR_{O_2} in indomethacin-treated animals suggests that the drug primarily inhibits prostacyclin synthesis in the vessel walls. Some support for this assumption is provided by the finding of Pickard et al. (1977a) that prostaglandins E_2 and $F_{2\alpha}$ reduce CBF and CMR_{O_2}

equally. As a working hypothesis, we may thus assume that prostacyclin reduces cerebrovascular tone and that "complete" (see below) inhibition of prostacyclin synthesis removes this influence.

2. CBF and CMR_{O_2} under hypercapnic conditions

Our results on hypercapnic animals are in close agreement with those of Pickard & MacKenzie (1973). In both series, indomethacin almost abolished the CBF response to increased CO_2 tensions but failed to alter CMR_{O_2} . In view of the results obtained it is tempting to conclude that changes in CO_2 tension exert their main effect on CBF by modulating prostaglandin (prostacyclin?) synthesis in the vessel walls. As stated, the relative changes in CBF during normo- and hypercapnia were similar. However, the true CO_2 responsiveness of the cerebral vessels is probably best expressed by the $\Delta\text{CBF}/\Delta\text{PCO}_2$ ratio. In uninjected animals the ratio was 0.088 and in indomethacin-injected ones it was $0.021 \text{ ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1}$. Thus, the drug reduced CO_2 responsiveness, so defined, to 25 percent of control (70% N_2O).

3. Dose response curves

The results demonstrating that indomethacin clearly affected CBF at a dose of $1 \text{ mg} \cdot \text{kg}^{-1}$ and showed maximal effect at $3\text{--}5 \text{ mg} \cdot \text{kg}^{-1}$ provide evidence that it acted via an inhibition of the fatty acid cyclo-oxygenase (Flower 1974). As shown in Fig. 6 there was a striking correspondance between the present dose response curves and those previously published by Abdel-Halim et al. (1978). However, although these authors calculated dose response curves from the percentage reduction of $\text{PGF}_{2\alpha}$ level in brain homogenates it seems less likely that the present results reflect inhibition of $\text{PGF}_{2\alpha}$ formation (see above). It will remain for further studies to establish the nature of the arachidonate metabolites that mediate effects on CBF revealed by the present experiments.

4. Time-dependent responses

The rapidity of action of i.v. indomethacin suggests that it acts in a tissue compartment which is in rapid diffusion equilibrium with blood. It is tempting to assume that this is a vascular compartment, e.g. the site of prostacyclin synthesis. However, this will remain an assumption until specific blockers

become available.

5. Regulation of CBF

Until quite recently, it was generally assumed that the coupling of cerebral metabolism and blood flow was exclusively mediated by products of metabolism such as H^+ , K^+ and adenosine. As reviewed elsewhere (Nilsson et al. 1978) it now seems that none of these factors qualify as a general coupling factor in many conditions associated with an increased CBF. These include hypoxia, epileptic seizures, hypoglycemia and adrenaline-induced hyperemia. The present results, and those previously reported by Pickard & MacKenzie (1973), demonstrate that hypercapnia represents another situation in which it is possible to partly dissociate changes in extracellular pH from changes in CBF. Undoubtedly, prostaglandin-like substances must be added to the list of putative coupling factors. However, inhibition of prostaglandin formation does not affect the increase in CBF during hypoxia (Sakabe & Siesjö 1979), bicuculline-induced seizures (Ingvar, Nilsson & Siesjö 1980) or hypoglycemia (Nilsson, Agardh, Ingvar & Siesjö, in preparation). In view of these results, it seems less fruitful to look for coupling factors that are common to all conditions considered.

The indomethacin-induced inhibition of the CO_2 response indicates powerful vasomotor modulating effects of prostaglandins and related compounds on the cerebral vasculature, although the exact features of these mechanisms remain to be clarified. It has been suggested that CBF, particularly its CO_2 response, is regulated via brain stem centers (Shalit et al. 1967, Fenske et al. 1975, Capon 1975), which might be related to the brain stem center mediating the ventilatory response (Wright, Keele & Neil 1971). In order to study whether or not indomethacin affects the ventilatory response to hypercapnia, we anesthetized 3 rats with halothane 1% in N_2O 70%/O₂ 30% via a tight mask, and inserted a venous catheter. An elastic strain gauge transducer was attached around the chest and connected to a bridge instrument allowing continuous recording of respiratory movements. At steady state 10% CO_2 was repeatedly administered for 30 s periods. Although indomethacin $10\text{ mg}\cdot\text{kg}^{-1}$ i.v. induced a slight hyperventilation (see also Results: 1) it failed to

alter the ventilatory response to CO₂ inhalation. These findings do not exclude that the CO₂ effect on CBF could be mediated via a brain stem center, but demonstrate that the brain stem mechanisms mediating the ventilatory response are different from the mechanisms involved in the vasomotor response to CO₂. The I-CBF results, indicating a very uniform effect of indomethacin on the vascular bed, and the well defined dose-response curve of indomethacin obtained, further indicate a diffuse, peripheral mechanism for the CO₂ response and the indomethacin effect.

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Effects of indomethacin on cerebral blood flow and oxygen consumption in barbiturate-anesthetized normocapnic and hypercapnic rats.

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ABSTRACT

Although results obtained in baboons and rats have demonstrated that the fatty acid cyclo-oxygenase inhibitor indomethacin reduces CBF under control conditions, and markedly attenuates the CBF response to hypercapnia, nonconfirmatory results have been obtained in rabbits and cats. Since these latter studies were carried out under barbiturate anesthesia we tested the effect of indomethacin ($10 \text{ mg} \cdot \text{kg}^{-1}$) on CBF and CMR_{O_2} in rats anesthetized with $150 \text{ mg} \cdot \text{kg}^{-1}$ of phenobarbital.

At normocapnia the barbiturate reduced CBF, measured with a ^{133}Xe modification of the Kety-Schmidt technique, to about 50 per cent of nitrous oxide control values, previously determined with similar technique. At this CBF level indomethacin induced a very small, albeit highly significant decrease in CBF. We suggest that a reduction of this magnitude will escape detection with some CBF techniques in current use. Indomethacin induced a highly significant decrease in CBF during hypercapnia, demonstrating that the barbiturate does not obliterate the effect of indomethacin on CO_2 responsiveness. The magnitude of the reduction in CO_2 response was so large that it should be detected with most methods for measuring CBF.

A comparison with previous data on animals under 70 per cent N_2O demonstrated that phenobarbital reduced the CO_2 responsiveness, defined as the ratio $\Delta\text{CBF}/\Delta\text{P}_{\text{CO}_2}$, to 39 per cent of that observed under nitrous oxide analgesia. With both types of anesthesia, indomethacin curtailed the CO_2 responsiveness 4- to 5-fold.

In spite of the fact that the problem has been studied over many decades, the factors that adjust the cerebral blood flow (CBF) to functional and metabolic demands have not yet been defined. Recent results indicate that some of these missing coupling factors may be provided by products of the fatty acid cyclo-oxygenase, i.e. by prostaglandins and related substances. The evidence comes from studies in which the cyclo-oxygenase was inhibited by indomethacin, a potent inhibitor of the enzyme in brain and other tissues (see Flower 1974, Wolfe et al. 1976, Abdul-Halim et al. 1978).

The original report describing an effect of indomethacin on CBF was published by Pickard and MacKenzie (1973) who administered the drug by intravenous ($10 \text{ mg} \cdot \text{kg}^{-1}$) or intracarotid ($0.04\text{--}0.2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) routes in phencyclidine- N_2O anesthetized baboons, measuring CBF with a $^{133}\text{xenon}$ clearance technique. The authors found that the drug reduced control CBF by an average of 38 per cent, and that it markedly attenuated the CBF response to induced hypercapnia, without affecting the cerebral metabolic rate for oxygen (CMR_{O_2}). Subsequent studies from the same group demonstrated that indomethacin failed to affect the autoregulatory ability of the cerebral vasculature in response to changes in perfusion pressure (Pickard et al. 1977a). Since experiments with intracarotid infusion of PGE_2 and $\text{PGF}_{2\alpha}$, without or with prior osmotic disruption of the blood-brain barrier, showed that these prostaglandins reduced both CBF and CMR_{O_2} (Pickard et al. 1977b), it seemed that indomethacin must have inhibited the formation of another cyclo-oxygenase product. Recent results (Pickard et al. 1980) indicate that this product is prostacyclin, a 'prostaglandin' which has been shown to be localized to vascular tissue and to mediate vasodilatation in other tissues than the brain (Moncada and Vane 1978, Jarman et al. 1979, Chapleau et al. 1980).

Studies from our own laboratory have confirmed and extended the original observations of Pickard and MacKenzie (1973). Thus, Sakabe and Siesjö (1979), working on rats under N_2O analgesia and measuring CBF with a $^{133}\text{xenon}$ modification of the Kety and Schmidt technique, found that indomethacin ($10 \text{ mg} \cdot \text{kg}^{-1}$ i.v.) decreased CBF to 50 percent of control, and reduced CBF

under hypercapnic conditions (PaCO_2 about 80 mm Hg) to about 30 per cent of hypercapnic control, CMR_{O_2} being unchanged both at normo- and hypercapnia. Since indomethacin did not curtail the circulatory response to hypoxia, a general unreactivity of the cerebral vasculature could be excluded. The reduction in CBF induced by indomethacin was verified with two completely different CBF techniques (Dahlgren et al. 1981) based on autoradiographic assessment of local CBF with ^{14}C -iodoantipyrine as the diffusible tracer and continuous cerebral venous out-flow measurements, respectively. In that study, local CBF at normocapnia was reduced to 40-80 per cent of control, with cerebral cortical structures having flow rates of 40-50 per cent of control. A comparison showed that the relative (i.e. percentual) reduction in CBF, brought about by indomethacin, was similar in normocapnia and hypercapnia (see below). Two further results should be noted. First, effects on CBF were observed at an indomethacin dose of $1 \text{ mg} \cdot \text{kg}^{-1}$, and maximal effects were observed at an indomethacin dose of $3\text{-}5 \text{ mg} \cdot \text{kg}^{-1}$. Second, following i.v. administration of indomethacin CBF began decreasing already within 10 sec and maximal effects were noted after about 2 min. These results indicate that the effect of indomethacin was related to cyclo-oxygenase inhibition (see Flower 1974) and that the drug acted on an enzyme complex in fast diffusion equilibrium with blood (the vascular wall?).

Although the results obtained in baboons and rats appear convincing, non-confirmatory results have appeared. Cuypers et al. (1978), measuring relative changes in CBF with a thermoclearance technique in rabbits, reported that indomethacin neither reduced 'resting' CBF, nor attenuated the response to hypercapnia. Similar results were reported by Wei et al. (1980) who measured pial artery caliber in cats. The experiments can be criticized on the grounds that none of these techniques give quantitative measures of blood flow (see Discussion). However, an even more important factor may be the barbiturate anesthesia used since barbiturates by themselves reduce CBF and attenuate the circulatory response to hypercapnia (Fujishima et al. 1971, Grubb et al. 1974).

The present experiments were undertaken to assess the effect

of indomethacin on CBF and CMR_{O_2} under normocapnic and hypercapnic conditions in barbiturate-anesthetized animals. To that end, ventilated rats maintained on phenobarbital anesthesia were exposed to hypercapnia, with or without prior administration of indomethacin in a dose of $10 \text{ mg} \cdot \text{kg}^{-1}$.

MATERIALS AND METHODS

1. Materials

Phenobarbital (Fenemal^(R), $200 \text{ mg} \cdot \text{kg}^{-1}$, ACO, Stockholm) and d-tubocurarine (Tubocurarine^(R), $3 \text{ mg} \cdot \text{ml}^{-1}$, Vitrum, Stockholm) were used in stock solutions as supplied. Heparin (Vitrum, Stockholm) was diluted in Krebs solution to give a concentration of $300 \text{ IU} \cdot \text{ml}^{-1}$. Indomethacin (Confortid^(R), Dumex, Helsingborg) was dissolved in distilled water to give a concentration of $10 \text{ mg} \cdot \text{ml}^{-1}$. Thus, it was not necessary to use bicarbonate-containing solutions. pH in the solution was about 7.70. Only freshly prepared solutions were used. $^{133}\text{Xenon}$ gas was provided by AB Atomenergi, Studsvik.

2. Animals

All experiments were performed on male Wistar rats (SPF strain, Møllegaards Avlslaboratorium, Copenhagen) weighing 305 to 460 g. The animals had free access to tap water and food pellets (Astra-Ewos, Södertälje) until operation.

3. Operative and sampling techniques

The rats were anaesthetized by intraperitoneal injection of phenobarbital $150 \text{ mg} \cdot \text{kg}^{-1}$. When unresponsive, the animals were tracheotomized and artificially ventilated with N_2/O_2 (70:30) by a Starling type respirator. Immobilization was accomplished by d-tubo-curarine $1.5 \text{ mg} \cdot \text{kg}^{-1}$ i.v. Body temperature was controlled and kept close to 37°C by means of a heating bulb. Blood gas and pH measurements (Eschweiler and Co., Kiel, and Radiometer, Copenhagen) were corrected for any deviation from this temperature. One brachial artery, and a femoral artery and vein were cannulated to accomplish arterial sampling for ^{133}Xe -determination during desaturation, continuous blood pressure recording, anaerobic sampling for blood gas analyses, injection of drugs and transfusion of donor blood (see below). The skull was exposed by a small incision and a

hole in the skull bone drilled 7-8 mm caudal to the bregma giving access to cerebrovenous blood from the superior sagittal sinus. Heparin $300 \text{ IU} \cdot \text{kg}^{-1}$ was given i.v. at the end of the operation. The animals were then allowed a steady state period of 30 min before experimental procedures were instituted (see below). During that period checks were made that the PaCO_2 was in the range of 35-40 mm Hg, and that the PaO_2 exceeded 90 mm Hg.

4. Measurement of CBF and CMRO_2

CBF was measured using a modification of the Kety-Schmidt technique with ^{133}Xe gas as the diffusible tracer (Norberg and Siesjö 1974, Berntman et al. 1979). The animals were saturated for 20 min with ^{133}Xe tracer gas added to the insufflated gas mixture. Desaturation curves were obtained by repeated sampling of arterial and cerebrovenous blood and the area between the curves was calculated by means of the trapezoid rule. A ^{133}Xe partition coefficient of 0.83 was used to calculate CBF. Total oxygen content was measured according to Borgström et al. (1974) in 4 pairs of arterio-cerebrovenous samples, two taken just prior to and two during the first minutes of desaturation. This gave a value for the arterio-venous difference in oxygen content (AVDO_2) that, multiplied by the CBF, gave CMRO_2 . The experiment was discarded if values for AVDO_2 prior to and during flow measurement differed by more than 15 per cent.

In order to avoid a fall in blood pressure during sampling of arterial and venous blood, fresh donor blood was intermittently infused (3-5 ml).

5. Design of experiments

Four conditions were studied: normocapnia and hypercapnia with and without indomethacin. In each of these four groups of animals indomethacin or an equivalent amount of saline was injected at the end of the steady state period and CBF was measured 45 min later. In the two hypercapnia groups, 5-6 per cent CO_2 was added to the insufflated gas mixture 15 min after indomethacin injection, i.e. 30 min prior to CBF measurements. Since only one CBF - CMRO_2 determination was made in each animal, the length of anaesthesia and the duration of indometha-

cin action were similar in all animals.

6. Calculations

In calculating statistical differences, homogeneity of variance was first tested. Depending on the outcome of this test statistical calculations were performed using the Student's t-test, or the Mann-Whitney U-test.

RESULTS

1. Physiological parameters

Mean values for body temperature and mean arterial blood pressure as well as for arterial P_{O_2} , P_{CO_2} , and pH are given in Table 1.

Table 1. Physiological parameters for rats under barbiturate anesthesia (phenobarbital, 150 mg·kg⁻¹) under normo- and hypercapnia, with or without indomethacin (10 mg·kg⁻¹).

Experimental groups n=6	Temp °C	MABP mm Hg	P_{aO_2} mm Hg	P_{aCO_2} mm Hg	pH
1. Normocapnia	37.1 ±0.2	117 ±4	109 ±2	37.1 ±0.6	7.40 ±0.02
2. Hypercapnia	37.0 ±0.1	126 ±3	113 ±2	79 ±2***	7.17 ±0.02***
3. Normocapnia + indomethacin	37.3 ±0.1	116 ±5	113 ±6	37.1 ±1.1	7.36 ±0.01
4. Hypercapnia + indomethacin	37.0 ±0.2	121 ±2	117 ±3	83 ±2***	7.15 ±0.01***

Values are given as mean ± SE. * denotes $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

Injection of indomethacin did not cause any significant differences.

MABP = mean arterial blood pressure.

Body temperature and arterial P_{O_2} were similar in all four groups. Both normocapnic groups had similar arterial P_{CO_2} and pH values, and hypercapnia induced similar changes in all these variables. It can thus be excluded that results on CBF (or CMR_{O_2}) were influenced by differences in the physiological state of the animals.

2. CBF and CMR_{O_2}

Previous results from the laboratory suggest that the Kety and Schmidt technique, modified for the rat, yields accurate values for CBF and CMR_{O_2} at normal and increased flow rates (see Siesjö et al. 1980). In the present study, markedly reduced CBF values were encountered, as compared with nitrous oxide anaesthesia. Fig.1 shows the results of a truly typical

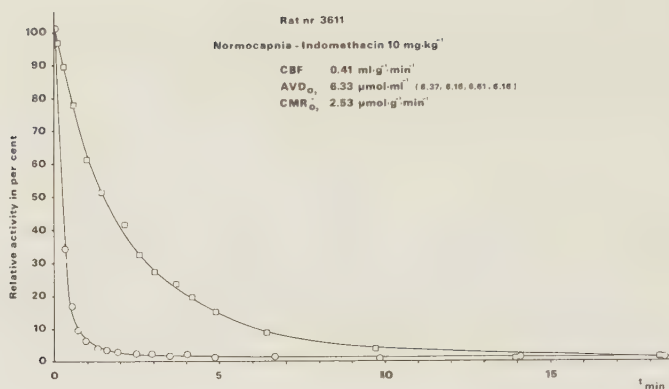


FIG.1. Arterial (circles) and cerebrovenous (squares) clearance curves for $^{133}\text{xenon}$ under normocapnia in a low flow condition induced by barbiturate (phenobarbital, $150 \text{ mg}\cdot\text{kg}^{-1}$) and indomethacin ($10 \text{ mg}\cdot\text{kg}^{-1}$).

CBF measurement at flow rates of $0.5 \text{ ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$, or less. The equalisation of arterial and cerebrovenous $^{133}\text{xenon}$ activities within 15 min, and the constancy in the arteriovenous difference in oxygen content, suggest that accurate values for CBF and CMR_{O_2} were obtained.

Data for arterial oxygen content (Ca_{O_2}), arteriovenous difference in oxygen content (AVD_{O_2}), CBF and CMR_{O_2} are given in Table 2. Since Pa_{O_2} values exceeded 100 mm Hg (see Table 1 above) we can conclude from the Ca_{O_2} values that the hematocrit was similar in all groups. The CBF and CMR_{O_2} values measured in normocapnic animals are typical for barbiturate animals (see Discussion). Hypercapnia induced a 3.6-fold increase in CBF without causing any significant change in CMR_{O_2} . Due to this dissociation of CBF and CMR_{O_2} , AVD_{O_2} decreased markedly.

Administration of indomethacin to normocapnic animals reduced CBF by 20 per cent. Since the standard errors were small, this represented a highly significant change ($p < 0.01$). As in animals maintained on 70 per cent N_2O (Sakabe and Siesjö

Table 2. Arterial oxygen content (CaO_2), cerebral arteriovenous oxygen difference (AVDO_2), cerebral blood flow (CBF) and cerebral metabolic rate for oxygen (CMRO_2) for rats under barbiturate anesthesia (phenobarbital, 150 $\text{mg}\cdot\text{kg}^{-1}$) under normo- and hypercapnia, with or without indomethacin (10 $\text{mg}\cdot\text{kg}^{-1}$).

Experimental groups n=6	CaO_2 $\mu\text{mol}\cdot\text{ml}^{-1}$	AVDO_2 $\mu\text{mol}\cdot\text{ml}^{-1}$	CBF $\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$	CMRO_2 $\mu\text{mol}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$
1. Normocapnia	10.87 $\pm$ 0.34	5.02 $\pm$ 0.19	0.55 $\pm$ 0.02	2.75 $\pm$ 0.13
2. Hypercapnia	10.21 $\pm$ 0.26	1.63 $\pm$ 0.18***	1.97 $\pm$ 0.20***	3.03 $\pm$ 0.15
3. Normocapnia + indomethacin	10.58 $\pm$ 0.25	5.83 $\pm$ 0.28†	0.43 $\pm$ 0.03††	2.47 $\pm$ 0.15
4. Hypercapnia + indomethacin	10.22 $\pm$ 0.23	3.89 $\pm$ 0.32†††	0.73 $\pm$ 0.03†††	2.78 $\pm$ 0.17

Values are given as means $\pm$ SE. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ when comparing the effects of hypercapnia (1 versus 2, 3 versus 4). † $p < 0.05$, †† $p < 0.01$, and ††† $p < 0.001$ when comparing the effects of indomethacin (1 versus 3, 2 versus 4).

1979, Dahlgren et al. 1981) CMRO_2 remained unchanged. Although CBF during hypercapnia was only 0.73 $\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$, this represented an 1.7-fold increase from control. Thus, although indomethacin reduced absolute CBF during hypercapnia to one third of hypercapnic control, the drug did not completely obliterate the CO_2 response. Again, CMRO_2 remained unchanged.

DISCUSSION

The main results of this study can be summarized as follows. First, in animals maintained on barbiturate anaesthesia indomethacin leads to a reduction in CBF which is so moderate that it could escape detection with any but the most reproducible techniques. Second, barbiturate anaesthesia does not prevent indomethacin from curtailing the circulatory response to induced hypercapnia. Since hypercapnic flow rates in control and indomethacin-injected animals differed by a factor of almost 3 ($p < 0.001$) it remains an unsolved question why the effect was not observed by previous workers (Cuyppers et al. 1978, Wei et al. 1980). Based on the present results, and on previous observations of the effect of indomethacin on pial vessels and isolated middle cerebral artery strips (Vlahov and

Betz 1974, Pickard et al. 1976) it seems justified to conclude that changes in pial artery diameter, or in heat clearance, do not truly reflect alterations in nutritional blood flow following indomethacin administration. Thus, clear effects of indomethacin on the CO₂ responsiveness have been observed in two species by workers who have used quantitative CBF techniques (see Introduction). These studies have also revealed that maximal effects are observed with doses as low as 3 mg·kg⁻¹, and that the effect is rapid in onset. It should also be remarked that the effect persists for at least 2-3 hrs (unpublished observations).

It was noted above that the relative increase in CBF during hypercapnia was not much affected by indomethacin in animals maintained on 70 per cent N₂O. However, it has been observed before that the CO₂ sensitivity, expressed as absolute change in CBF per mm Hg ($\Delta\text{CBF}/\Delta\text{P}_{\text{CO}_2}$), is reduced in barbiturate anesthesia, and that this reduction bears some relationship to CMR_{O₂} (Fujishima et al. 1971). A similar reduction in CO₂ sensitivity has been noted in the baboon, in which chloralose anaesthesia (as compared to Sernylan anaesthesia) reduced both CMR_{O₂} and the CO₂ responsiveness (Sándor et al. 1977). It is clear that the CO₂ responsiveness, so calculated, is reduced by indomethacin although the drug does not decrease CMR_{O₂} significantly (Pickard and MacKenzie 1973, Sakabe and Siesjö 1979, Dahlgren et al. 1981).

The present results, and those previously obtained in animals under 70 per cent N₂O, allow a comparison of CO₂ responsiveness in N₂O and phenobarbital-anaesthetized animals, as well as evaluation of the effects of indomethacin (Fig.2). We recognize the difficulty in calculating $\Delta\text{CBF}/\Delta\text{P}_{\text{CO}_2}$ values from a curve drawn between only two points. However, assuming a roughly linear relationship between PaCO₂ and CBF in the range of 40 to 80 mm Hg we calculated approximate indices. As the results show, phenobarbital anaesthesia reduced $\Delta\text{CBF}/\Delta\text{P}_{\text{CO}_2}$ to about 40 per cent of the 70 per cent N₂O value. Furthermore, indomethacin reduced CO₂ responsiveness similarly in N₂O- and phenobarbital-anaesthetized animals (4- to 5-fold).

The present results give information on two further issues,

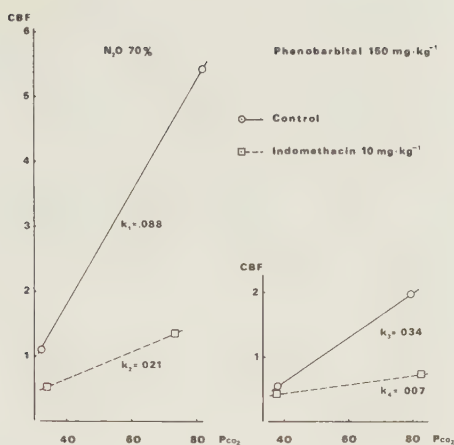


FIG.2 CO₂ responsiveness under N₂O (70%) analgesia and phenobarbital (150 mg·kg⁻¹) anaesthesia expressed as $\Delta\text{CBF}/\Delta\text{P}_{\text{CO}_2}$. K₁ and K₃ = slope for $\Delta\text{CBF}/\Delta\text{P}_{\text{CO}_2}$ line without indomethacin. K₂ and K₄ = slope for $\Delta\text{CBF}/\Delta\text{P}_{\text{CO}_2}$ line with indomethacin (10 mg·kg⁻¹).

one of which represents an unresolved controversy. The first issue concerns the cerebral metabolic effects of indomethacin. As stated, all studies have shown that indomethacin reduces CBF at constant CMR_{O₂}. However, inspection of the present data, and those reported by Dahlgren et al. (1981), shows that in all groups studied (normo- and hypercapnic animals) the mean value for CMR_{O₂} was lower in indomethacin-treated animals. In order to test the possibility that a true decrease in CMR_{O₂} remained undetected due to the small groups studied we calculated all individual control and indomethacin CMR_{O₂} values in percent of the mean and pooled the percentage figures for all four groups (phenobarbital and N₂O-anaesthetized animals at normal or increased CO₂ tensions). A statistical comparison showed that indomethacin causes a reduction in CMR_{O₂} by 13 per cent ($p < 0.01$). The indomethacin treated animals were 29, compared with 27 appropriate controls.

The second and controversial issue referred to concerns the effect of hypercapnia on CMR_{O₂}. As reviewed elsewhere (Siesjö 1980), various groups have reported decreased, unchanged, or increased CMR_{O₂} values in hypercapnia. Our own results, which

have demonstrated that hypercapnia of the degree studied presently increases CMR_{O_2} , have been criticised on the grounds that calculations of CMR_{O_2} at very high flow rates may be in error (Artru and Michenfelder 1980). In order to explore whether pooling of data gives information on this matter we performed the same statistical manoeuvre as for the effect of indomethacin on CMR_{O_2} , using control and indomethacin treated animals with N_2O or phenobarbital anaesthesia. The results showed that hypercapnia gives rise to an increase in CMR_{O_2} by 12 per cent ($p < 0.05$). The hypercapnic groups comprised 31 animals compared with 25 appropriate controls.

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The effect of indomethacin on local cerebral blood flow
in awake, minimally restrained rats

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ABSTRACT

Experiments on two animal species have shown that indomethacin markedly reduces cerebral blood flow (CBF) with little or no effect on cerebral oxygen consumption (CMR_{O_2}). Pickard and MacKenzie (1973; see also Pickard et al., 1977), working on normocapnic baboons under phencyclidine- N_2O anesthesia, found that indomethacin ($10 \text{ mg}\cdot\text{kg}^{-1}$ i.v.) reduced CBF by an average of 38%. Confirmatory results have been reported in rats maintained artificially ventilated on 70% N_2O (Sakabe and Siesjö, 1979; Dahlgren et al., 1981). In that species, indomethacin in doses of $3\text{--}10 \text{ mg}\cdot\text{kg}^{-1}$ reduced CBF, as measured by a modified Kety-Schmidt technique with sampling of venous blood from the superior sagittal sinus, by about 50%. Corresponding regional data, as obtained with an autoradiographic ^{14}C -iodoantipyrine technique (Sakurada et al., 1978), showed that local CBF was reduced to between about 40 and 70% of control (Dahlgren et al., 1981).

While it is clear that phenobarbital anesthesia, which by itself reduces CBF, markedly attenuates the circulatory effect of indomethacin (Dahlgren and Siesjö, submitted for publication) the question remains whether immobilization and/or nitrous oxide (or phencyclidine) influence the response. The question is pertinent since autoradiographic results obtained in immobilized animals under 70% N_2O show a reduction in CBF of cerebral cortical areas to 40–50% of control (Dahlgren et al., 1981). By ordinary standards such a reduction in CBF should be close to that associated with signs of tissue hypoxia. One previous finding indicates that nitrous oxide has but a small influence on the CBF response to indomethacin. Thus, a similar reduction in CBF was noted in animals ventilated on 70% N_2 and 30% O_2 (Sakabe and Siesjö, 1979; Dahlgren et al., 1981). However, since the animals were paralyzed and adrenalectomized, the results do not conclusively prove the point.

In the present experiments we measured local CBF in awake, minimally restrained rats following administration of indomethacin in a dose of $10 \text{ mg}\cdot\text{kg}^{-1}$. Although the results confirm the reduction in CBF induced by the drug they clearly show that this reduction is enhanced in ventilated animals maintained on 70% N_2O . In the following we will refer to this

group by using the term "N₂O anesthesia".

MATERIALS AND METHODS

The present article is based on six rats that were given indomethacin in a dose of 10 mg.kg⁻¹ i.v. 45 min prior to l-CBF measurement. The experiments were performed at a time when the same group of workers evaluated l-CBF in awake animals breathing either air or 70-80% N₂O in O₂ (Dahlgren, Ingvar, Yokoyama & Siesjö, submitted for publication). Since the techniques were identical the air-breathing animals of that material was used as the control group in the present study. The previous communication gives details of methods and analytical techniques. In summary, male Wistar rats (310-380 g) were allowed food pellets and tap water until the day of the experiments. In the late afternoon, catheters were inserted in a tail artery and a tail vein under halothane anesthesia, and the base of the tail was infiltrated with a local anesthetic. A thermistor probe was inserted in the rectum for measurements of body temperature. After preparation, while still asleep, the animals were placed in a Perspex cage that was continuously flushed with room air. After 30 min, indomethacin was injected i.v. (about .35 ml solution). Fortyfive min later, ¹⁴C-iodo-antipyrine was infused i.v. during 45 sec and the animals were then decapitated. During the 45 sec period, 11-14 arterial samples were collected for measurements of ¹⁴C-activity. The tissue activity was determined in 22 defined brain structures with quantitative autoradiography.

As stated, the control animals were treated identically except for the injection of indomethacin. Since the drug was dissolved in distilled water, and since the catheters were repeatedly flushed with saline it was deemed unnecessary to match the experimental group to one injected with the same amount of vehicle.

Student's t-test was used for calculation of significance in differences between groups.

RESULTS

Physiological parameters in the indomethacin-injected animals were similar to those of the control group. Thus, means $\pm$ S.E.M. for body temperature, arterial blood pressure, PCO_2 , PO_2 and pH were $37.6 \pm 1^\circ C$, 111 ± 2 mm Hg, 41.6 ± 0.9 mm Hg, 80 ± 3 mm Hg, and 7.42 ± 0.01 , respectively.

In table 1, which gives the l-CBF data, the structures analysed have been arranged in the following groups: motor, sensory, limbic, and hypothalamus. All structures showed a highly significant ($p < 0.001$) and highly consistent decrease in l-CBF in animals given indomethacin. As the data demonstrate, l-CBF was reduced to between 55 and 75% of control. The least pronounced reduction in l-CBF (25%) was observed in two cortical structures (parietal and sensorimotor) and the most pronounced one (45%) in hypothalamus, septal nuclei, and amygdala. Corti-

Table 1. l-CBF values in $ml \cdot g^{-1} \cdot min^{-1}$ for 22 brain structures in awake animals with and without indomethacin ($10 \text{ mg} \cdot kg^{-1}$).

	Control	Indomethacin $10 \text{ mg} \cdot kg^{-1}$
Frontal cortex	1.20 ± 0.05	0.80 ± 0.02
Nc ruber	1.53 ± 0.05	0.95 ± 0.04
Striatum	1.38 ± 0.04	0.99 ± 0.03
Substantia nigra	1.11 ± 0.05	0.75 ± 0.04
Cerebellum	1.44 ± 0.07	0.92 ± 0.02
Sensory-motor cortex	1.46 ± 0.06	1.09 ± 0.04
Parietal cortex	1.52 ± 0.06	1.12 ± 0.03
Thalamus	1.46 ± 0.08	1.01 ± 0.03
Nc anterior thalami	1.67 ± 0.04	1.19 ± 0.04
Nc ventral posterior thalami	1.75 ± 0.09	1.25 ± 0.04
Visual cortex	1.26 ± 0.04	0.84 ± 0.02
Lat. geniculate body	1.44 ± 0.07	1.00 ± 0.03
Sup. colliculus	1.75 ± 0.05	1.12 ± 0.04
Auditory cortex	2.18 ± 0.08	1.27 ± 0.03
Med. geniculate body	1.93 ± 0.05	1.19 ± 0.04
Inf. colliculus	3.16 ± 0.07	2.12 ± 0.14
Oliv. superior	2.31 ± 0.10	1.33 ± 0.09
Hippocampus	1.00 ± 0.03	0.66 ± 0.03
Amygdala	1.15 ± 0.09	0.63 ± 0.02
Septal nc	1.27 ± 0.07	0.68 ± 0.02
Habenula	1.75 ± 0.08	1.22 ± 0.04
Hypothalamus	1.27 ± 0.06	0.70 ± 0.02

Values are given as means $\pm$ S.E.M. l-CBF values for indomethacin treated animals are all significantly reduced ($p < 0.001$) as compared to controls.

cal structures, though, were not uniformly affected since two of them (frontal and visual cortex) had reductions less than 30%, and one (auditory cortex) a reduction by 40%.

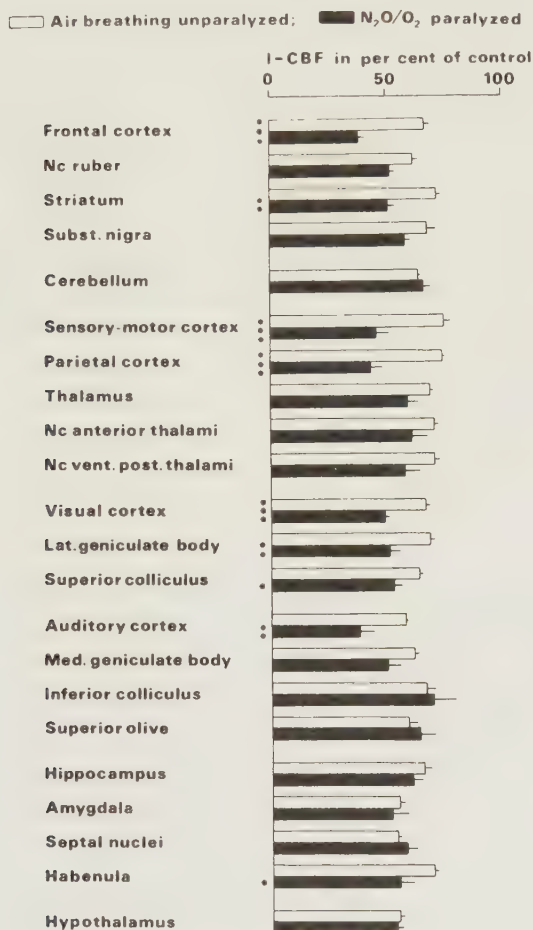


Fig. 1. The effect of indomethacin upon 1-CBF in 22 brain structures for air breathing, unparalyzed and N₂O/O₂ ventilated, paralyzed rats. Values are expressed in per cent of respective controls as means $\pm$ S.E. * denotes $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$, respectively for differences between groups.

Since the present results demonstrate somewhat less pronounced reductions in l-CBF than those observed in animals on N₂O anesthesia, a direct comparison is warranted. In fig. 1, the percentage reduction in l-CBF from control has been compared for awake animals (present material) and for those on N₂O anesthesia (data from Dahlgren et al., 1981). In about half of the structures, the relative decreases in CBF were similar. However, in some other structures animals on N₂O anesthesia appeared to show a more pronounced reduction in l-CBF and, in nine structures (cerebral cortical areas, the striatum, lateral geniculate body, superior colliculus, the habenula plus the striatum) the differences were highly significant.

In view of the fact that N₂O anesthesia affects CBF in several structures (see below) it is also of interest to compare absolute CBF figures in awake and N₂O-anesthetized animals given indomethacin. Such a comparison shows that in all structures analysed l-CBF was 10-20% lower in animals under N₂O anesthesia than in those that were awake (data not shown).

DISCUSSION

The present results, that were obtained in awake and minimally restrained animals showing no signs of stress or discomfort, corroborate previous results showing that indomethacin markedly reduces CBF (see Introduction). The data are also in accordance with a previous study in conscious rabbits in which global CBF, as measured with a microsphere technique, was reduced by 25% following i.v. indomethacin in a dose of 20 mg·kg⁻¹ (Bill, 1979). In the present study l-CBF was reduced to between 55 and 75% of control. A comparison with previous results, obtained in animals ventilated on 70% N₂O, shows that N₂O anesthesia accentuates the effect of indomethacin on CBF, at least in a number of cerebral structures. At first sight, the comparison between absolute CBF figures in awake and N₂O-anesthetized animals given indomethacin indicates that this "interaction" between indomethacin and N₂O anesthesia causes l-CBF to fall by 10-20% in all structures. However, such a comparison does not take the fact into account that N₂O anesthesia by it-

self affects I-CBF. It seems warranted, therefore, to discuss details of the proposed interaction.

As described in a previous communication (Dahlgren, Ingvar, Yokoyama & Siesjö, submitted), a comparison with results obtained in awake rats shows that N₂O anesthesia affects CBF in some structures. In none of the structures analysed, except the striatum, did N₂O anesthesia increase CBF significantly. However, in four of the five cortical structures analysed the mean CBF values exceeded awake control values by 15-35%, suggesting that N₂O anesthesia tends to increase CBF somewhat in cerebral cortical structures. A much clearer effect, though, was the reduction in CBF in several structures. This was most marked (a decrease by 35-40%) in the cerebellum, inferior colliculus, oliva superior, and septal nuclei, but was also significant in the hippocampus, amygdala, and hypothalamus. Clearly, these results show that N₂O anesthesia affects local CBF differently, the most pronounced differences being observed between, on one hand, most cerebral cortical areas and, on the other hand, cerebellum and limbic-hypothalamus structures.

In view of these results it would seem preferable to discuss the modulating influence of N₂O anesthesia on the response to indomethacin from the percentage figures given in Fig. 1. Clearly, these results demonstrate that N₂O anesthesia enhances the effect of indomethacin in structures that show an upheld (or increased) CBF during N₂O anesthesia, while it has little effect on the indomethacin response in which the N₂O anesthesia reduces CBF. In other words, in animals maintained on N₂O anesthesia the effect of indomethacin depends on the background pattern of CBF changes.

Many experimental approaches require immobilization and artificial ventilation. For these, it is necessary to establish circulatory and metabolic effects of drugs in the presence of immobilization, artificial ventilation and anesthetic/analgetic agents. However, it seems equally warranted to explore the effects elicited by such drugs in awake animals in the absence of stress, pain and/or discomfort. The present results, which provide such information, demonstrate that although indomethacin markedly reduces CBF under normocapnic conditions this

reduction does not exceed 45% of control in any of the structures analysed.

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